Philip S. Oshida

OPTIMUM TEMPERATURE FOR TISSUE CYTOSOL STORAGE

The metallothionein detoxification investigations conducted with scorpionfish, sea urchins, and mussels utilize the intracellular distribution of metals in animal tissues. In these studies, the relative distributions of metals bound to different cellular components of animal tissues are used as indicators of both metals detoxification and toxification. Interpretation of the results depends upon accurate assessment of intracellular distribution of metals in animals at time of sampling and dissection. To assure this accuracy, the temperature for storage of tissue cytosol had to minimize metal redistribution among intracellular metalloenzymes and other metalloproteins and cellular components during the time between dissection and analysis. This study indicated that -80°C was the most suitable temperature for storage of cytosol samples used in our detoxification research.

Redistribution of metals within cellular material is a common occurrence and is often due to interactions between the metal-containing enzymes and other proteins (Lehninger 1975). The rates of these enzyme-catalyzed reactions generally decrease with temperature within that temperature range in which the enzyme is stable and retains full activity. The rate of most enzymatic reactions is approximately halved for each 10°C decrease in temperature. Since enzyme activity is decreased with lowered temperature, it was predicted that the lowest temperature used in this study, -80°C, would be the storage temperature at which there is minimal activity, hence minimal redistribution of metals. The question that arose was whether there were significant differences between cytosols stored at -4, -20, and -80°C that would justify the increased costs of maintaining the lower temperature.

To determine the effects of temperature on metal redistribution during storage, sea urchin cytosol was stored for period of approximately 0, 4 and 8 weeks at each of the following temperatures: -4°C, -20°C, and -80°C. At the end of the appropriate time periods, the cytosols were separated by molecular weight using gel filtration. The molecular weight fractions were analyzed for their content of metals. This allowed comparison of metal levels in different molecular weight pools at different temperatures and after various storage durations. The stability at each temperature was assessed and the temperature that provided the most suitable metal-protein stability was utilized for storage of tissues and cytosol in all subsequent studies.

Purple sea urchins, *Strongylocentrotus purpuratus*, were collected intertidally from White Point on the Palos Verdes Peninsula, Los Angeles County, California. The sea urchins were dissected in the laboratory within 3 hours of collection.

For investigation into possible redistribution of metals during storage of cytosol, gonads from 30 sea urchins (both male and female) were combined and processed to cytosol in preparation for gel filtration chromatography.

The resulting cytosol was divided into seven aliquots. Each aliquot was put into a capped glass test tube. Two test tubes of cytosol were placed in each of three freezers (-4°, -20° and -80°C). The remaining cytosol was immediately applied to a Sephadex G-75 gel filtration column and the eluant fractions analyzed for metals. This "time zero" sample for the cytosol storage experiment was not frozen.

The gel filtration column separated the cytosol components according to molecular weight, and the eluant was collected as fractions using a standard fraction collector. All samples were applied to the same Sephadex G-75 column. Metal levels (copper, cadmium, and zinc) were determined in each fraction using flame or graphite furnace atomic absorption spectrophotometry. Metal levels were plotted and the peaks identified as the enzyme-containing pool (ENZ), the metallothionein-containing pool (MT) and the glutathione-containing pool (GSH) by the order of elution position. Metal levels in individual fractions of each pool were added and expressed as metal level in each pool per wet tissue weight.

After 4 and 8 weeks of storage, a test tube was removed from each freezer and the cytosol analyzed for metals in each protein pool. Since this was a preliminary experiment only single samples were analyzed.

DISCUSSION

The results indicated -80°C was the most appropriate temperature for storage of cytosol when tested with sea urchin gonads (Table 1). This temperature minimized the redistribution of zinc, copper, and cadmium among the molecular weight pools. At -80°C the approximate changes in total metals after 58 days of storage was 85 μg (18 percent) for zinc, 4 μg (22 percent) for copper, and 1 g (40 percent) for cadmium. The relative percentages of zinc, copper and cadmium in the ENZ, MT and GSH pools remained unchanged over the storage interval at this temperature.

Cytosol storage at -20°C may have been satisfactory but this could not be confirmed. The distribution of metals after cytosolic storage for 30 days at -20°C and -80°C were similar in most respects. However, the cytosol sampled after storage at -20°C showed a 44 percent decrease in total copper when compared to the "time zero"sample, and displayed a much different distribution pattern of copper than the "time zero" or -80°C stored cytosols. The 58-day sample which would have confirmed or denied this pattern of copper loss and redistribution was not analyzed because of a freezer failure on day 35. Storage temperatures between -20°C and -80°C were not tested because we did not have facilities available.

The storage of cytosol at -4°C does not appear feasible. Storage of sea urchin gonad cytosol at -4°C allowed major redistribution of the zinc. After 30 days storage at -4°C zinc (approx. 83-85 percent) which was in the metallothionein-containing pool at "time zero" was redistributed into the enzyme-containing and glutathione-containing pools. When assessment of the distributions of intracellular metals is an integral part of a study, as it is in the metallothionein/detoxification projects, such alterations of zinc patterns among molecular weight pools during storage are unacceptable.

Although absolute statements based on statistical analysis cannot be made due to lack of replicate sampling and analysis, a few salient features were observed. Storage of cytosol at -4°C

Table 1. Metals distribution in replicate sea urchin gonad cytosol samples stored for various durations and temperatures, showing the redistribution of zinc from the MT pool to the ENZ and GSH pools when stored at -20 and -4°C.

Storage		Cadmium (μg)				Copper (μg)				Zinc (μg)			
Temp (°C)	Duration (days)	Total	ENZ	MT	GSH	Total	ENZ	MT	GSH	Total	ENZ	MT	GSH
Never stored	0	2.28	.78(34)	1.25(55)	.24(11)	18	7(39)	10(56)	1(5)	477	272(57)	149(31)	56(12)
-80	30	1.55	.42(27)	.85(55)	.28(18)	14	5(36)	9(64)	N.D.**(0)	427	246(58)	136(32)	45(10)
-20	30	1.46	.37(25)	.87(60)	.22(15)	10	5(50)	5(50)	N.D. (0)	462	293(63)	86(19)	83(18)
-4	29	1.44	.39(27)	.87(60)	.18(13)	14	4(29)	10(71)	N.D. (0)	422	267(63)	22(5)	133(32)
-80	58	1.36	.37(27)	.75(55)	.24(18)	14	5(36)	9(64)	N.D. (0)	562	326(58)	148(26)	88(16)
-4	58	1.23	.47(38)	.66(54)	.10(8)	11	4(36)	6(54)	1(0)	467	299(64)	19(4)	149(32)

*For cadmium, copper or zinc, the number in the parentheses represents the percentage of the total amount of the metal that was measured in a pool at the specified time and after storage at the indicated temperature.
**N.D. means non-detectable. Detection limit was <1 μg/l.

allows for substantial zinc redistribution from the metallothionein-containing pools, the enzyme-containing and glutathione-containing pools. This was unsuitable for use in the metallothionein/detoxification studies. Cytosol storage at -20°C was similar to -80°C storage in many ways, but in this experiment, showed substantially decreased total copper levels and a changed distribution pattern after 30 days. The -80°C temperature appeared to be most suitable for storage. Changes in total cadmium, copper, and zinc were minimal, as were changes in distribution patterns.

Further study to investigate the effects of storage duration and temperature could be undertaken, but the more interesting, useful, and valuable aspects of contaminant detoxification by animals will be pursued, knowing that -80°C is an adequate method of cytosol storage. This storage temperature was used in all metallothionein/detoxification studies.

REFERENCE

Lehninger, A. L. 1975. Biochemistry, Second Edition. Worth Publishers. New York.