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notes on methodology

Preservation of lipid reesterifying enzymes in the microsomal fraction of the rat jejunum

A. Singh and J. B. Rodgers, Jr.*

Department of Medicine, Albany Medical College, Albany, New York 12208

Summary Studies were performed on methods of storage of rat jejunal tissue that would preserve activities of the lipid reesterifying enzymes, acyl CoA:monoglyceride acyltransferase and fatty acid CoA ligase. Storage at −80°C of microsomes prepared from jejunal mucosa or storage of lyophilized microsomes at −20°C was shown to preserve acyl CoA:monoglyceride acyltransferase very well for a matter of weeks. Preservation of fatty acid CoA ligase activity was adequate with either method, but results were not as good as for the transacylase enzyme.

During the past several years many studies have been performed on the lipid reesterifying enzymes from the small bowel of laboratory animals. These enzymes are labile, thus making it necessary to complete enzyme analyses on the same day samples are obtained. If a technique were developed for preservation of these enzymes, studies could be carried out with greater efficiency and flexibility. This report describes the results of several methods of preservation of these microsomal enzymes.

Mucosal samples from the jejunum of male Sprague-Dawley rats were obtained and microsomal fractions were prepared as previously described (1). Protein determinations on the microsomes were performed according to the method of Lowry et al. (2). Assays of acyl CoA:monoglyceride acyltransferase (MG-acyltransferase) and fatty acid CoA ligase (FA-CoA ligase) were performed by spectrophotometric methods as previously described (3). Duplicate determinations of enzyme activities were obtained. The average difference for duplicate assays was 5% for MG-acyltransferase and 4% for FA-CoA ligase.

A pool of microsomes from the jejuna of four rats was prepared, and MG-acyltransferase was assayed. 0.5-ml aliquots of microsomes in 0.154 M KCl–phosphate buffer, pH 7.4, were transferred to separate glass vials which were tightly stoppered; the vials were immediately stored at various temperatures: 4, −10, −20, and −80°C. At weekly intervals during the following 4 wk a

* To whom requests for reprints should be addressed.

TABLE 1. Activity of acyl CoA:monoglyceride acyltransferase of microsomes stored at various temperatures[a]

Temperature	Weeks			
	1	2	3	4
°C				
4	124	108	91	52
−10	123	124	110	97
−20	120	112	104	97
−80	136	128	136	132

[a] Activity of enzyme is expressed as nmoles/min/mg of microsomal protein. Original activity was 128 nmoles/min/mg.

vial stored at each temperature was allowed to thaw in ice water, and MG-acyltransferase activity was determined. Results shown in Table 1 indicate that activity was well maintained in samples stored at −80°C. However, loss of activity of 25–60% was observed after storage at higher temperatures.

To verify this observation the study was repeated on another pooled sample of jejunal microsomes. These were all stored at −80°C. Assays were performed at weekly intervals for 7 wk, and a final assay was performed after 11 wk of storage. During the first 7 wk the results were 89–100% of the original value (average value was 93%). After 11 wk the enzyme activity was still very well preserved (98% of the initial activity).

Investigations were also performed on lyophilized microsomes. These samples were prepared by transferring microsomal suspensions to ice-cold glass vials and freezing the samples in methyl alcohol–carbon dioxide snow. Lyophilization was then performed at a temperature between −25 and −40°C. After this process was completed the vials were tightly stoppered and stored at −20°C. For reconstituting lyophilized microsomes the vial was placed in ice water and the microsomes were resuspended in 1 ml of ice-cold 0.154 M KCl–phosphate buffer, pH 7.4.

To test this method of preservation, jejunal microsomes were obtained from four rats. These samples were not pooled. Assays of both MG-acyltransferase and FA-CoA ligase were performed on each sample without storage. Two 1-ml aliquots of each microsomal sample were lyophilized. One of each was immediately reconstituted in KCl buffer, and enzyme assays were repeated to determine whether the lyophilization process itself resulted in a loss of enzyme activity. The other lyophilized sample from each animal was stored for 3 wk at −20°C. What remained of each original microsomal sample was also stored separately at −80°C for 3 wk. After this period, enzyme activities were redetermined (Table 2). No loss of either enzymatic activity was observed after lyophilization alone. MG-acyltransferase activity was well maintained by both

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TABLE 2. Activities of microsomal lipid reesterifying enzymes after storage for 3 wk at −80°C and after lyophilization and storage at −20°C[a]

Type of Preparation	Weeks 1	2	3	4	Average	% of Original
MG-acyltransferase						
Original	132	150	166	146	149	
Lyophilized, not stored	135	143	159	150	147	99
Lyophilized, stored at −20°C	122	139	156	155	143	96
Stored at −80°C	151	146	153	145	149	100
FA-CoA ligase						
Original	131	128	126	124	127	
Lyophilized, not stored	135	124	119	106	121	95
Lyophilized, stored at −20°C	111	90	110	116	107	84
Stored at −80°C	118	108	104	112	111	87

[a] Activities of enzymes are expressed as nmoles/min/mg of microsomal protein. Four rats were used to prepare microsomes from the jejunum. The microsomal sample from each was processed and assayed separately.

methods of storage, but activities of FA-CoA ligase declined about 15%.

Attempts were also made to preserve mucosal samples for various periods. These samples were allowed to thaw in ice water, and then the microsomal fraction was prepared for enzyme assay. This method of storage even at −80°C proved to be much less effective at maintaining enzyme activity.

These studies demonstrate that it is possible to preserve microsomal lipid reesterifying enzyme activity from the jejunum at −80°C for a period of at least 11 wk. If facilities are not available for storage at this temperature, lyophilization and storage at −20°C seems to be a satisfactory alternative.

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