The influence of calcium chloride, adenosine triphospate and lysosomal enzymes on water holding capacity of myofibrillar proteins

Y. M. ELSHREK [1] AND M. E. BAILEY [2]

ABSTRACT

The influence of calcium chloride ($CaCl_2$), adenosine triphosphate (ATP) and lysosomal enzymes independently and in combinatin on water holding capacity (WHC) of beef myofibrillar proteins was studied. $CaCl_2$ or ATP decreased WHC at both pH 5.5 and 7.0. The WHC of samples treated with a mixture of $CaCl_2$ and ATP was less than that of untreated proteins. The WHC of samples treated with lysosomal enzymes was enhanced by adding ATP, $CaCl_2$ and a mixture of the two.

INTRODUCTION

WHC represents the potential water retention of a material in the presence of excess moisture (6). WHC is considered one of the most imporatnt features of meat quality, however; WHC of meat is closely related to flavor, color, tenderness and other features of meat quality (3). Tenderness of meat is presently rated as one of the most important quality attributes of meat by the average consumer. It appears to be sought sometimes at the expense of flavor and color of meat (4).

Storage of meat under refrigeration for short periods result in an increased tenderness, but the biochemical mechanisms involved in tenderization of meat during this aging period remain obscure. The most likely explanation for these changes is that myofibrillar proteins are hydrolyzed by certain proteolytic enzymes such as lysosomal enzymes.

The addition of ATP to the muscle increased its WHC. On the other hand, the addition of Ca^{++} caused dehydration of actomyosin (3).

The objective of this study was to find out the effect of $CaCl_2$ and ATP on the activity of lysosomal enzymes by using WHC as a tool to measure the lysosomal enzymes activity.

MATERIALS AND METHODS

Meat Samples:

Beef semimembranous muscles were used to prepare myofibrillar proteins. Muscles were removed within one-half hour postexagunation immediately after killing, eviscerating and washing.

[1] *Food Science Department, Al-Fateh University, Tripoli, S.P.L.A.J.*
[2] *Food Science and Nutrition Department. Univ. of Missouri, Columbia, U.S.A.*

Preparation of Proteins:

Myofibrillar proteins were prepared at 1.5 and 96hrs post-mortem of animals. The method used was that described by Briskey and Fukawaza (1). Protein concentration was determined by the biuret method described by Cooper (2).

Source of Enzyme: (10,000 × g)

Muscle lysosomal enzymes were isolated from, *crus laterale* of the diaphragm muscle as described by Parrish and Bailey (5).

WHC Determination:

Reactions were carried out in solutions prepared by adding five ml (125 mg protein) beef myofibrillar proteins suspended in borate-KC1 buffer (0.025 M KC1, 0.039 M potassium borate and 2 mM sodium azide, pH 7.0 with 0.1 M NaOH, with or without 0.5 ml beef lysosomal enzymes (5 mg protein) and the final volume adjusted to 10 ml with distilled water. The solutions were incubated at 4°C for 24 and 96 hrs. Following the reactions the samples were centrifuged at 2000 × g for 15 min. Supernatant was measured volumetrically. The following equation was applied to calculate water binding capacity (WHC %).

$$\text{WHC \%} = \frac{\text{10 ml protein solution - ml of supernate}}{\text{10 ml}} \times 100$$

RESULTS AND DISCUSSION

Results in Table 1 indicate that $CaCl_2$ or ATP decreased WHC at pH 5.5 and pH 7.0. The WHC of samples treated with a mixture of $CaCl_2$ and ATP was less than that of control myhofibrillar proteins.

Beef muscle lysosomal enzymes activity increased WHC at pH 5.5 and at pH 7.0 and even with added $CaCl_2$. Myofibrillar proteins bound more water when treated with lysosomal enzymes.

ATP enhanced lysosomal enzymes degradation of myofibrillar proteins as indicated by the increase in water binding. The influence of $CaCl_2$ on enzyme activity could not

Table 1 — Mean[a] water holding capacity of beef myofibrillar proteins after reaction at 4°C for 24 and 96 hr with lysosomal enzymes, $CaCl_2$ and ATP[b].

	WHC%			
	pH			
	ph 5.5		pH 7.0	
Treatments	24 h	96 h	24 h	96 h
Myofibrillar Protein (MFP)	45.55	46.75	65.67	65.67
MFP + $CaCl_2$	43.32	45.27	63.97	64.80
MFP + ATP	45.07	45.43	65.22	65.50
MFP + $CaCl_2$ + ATP	42.30	42.22	61.40	64.60
MFP + Enzyme	48.40	53.83	73.75	77.88
MFP + Enzyme + $CaCl_2$	50.98	54.50	65.63	75.12
MFP + Enzyme + ATP	49.60	51.08	77.10	81.90
MFP + Enzyme + $CaCl_2$ + ATP	45.03	45.12	61.52	61.07

[a] N = 6

[b] Proteins were incubated for 24 and 96 h at 4°C with 5 mM $CaCl_2$ and 5mM ATP.

be measured by water binding, because of direct decrease in WHC in the presence of calcium ions. Therefore, the conclusion of this study is that the WHC can be used as a measure of limited proteolysis, except in the presence of $CaCl_2$.

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تأثير كلوريد الكالسيوم، ثلاثى فوسفات الآدينوسين والأنزيمات الليسوسومية على قوة الحفظ المائى للبروتين الليفى

د. يوسف الشريك

م. ى. بيلى

المستخلص

درس تأثير كلوريد الكالسيوم، ثلاثى فوسفات الأدينوسين والأنزيمات الليسوسومية كل على حدة أو مجتمعة على قوة الحفظ المائى للبروتين الليفى. . انخفضت قوة الحفظ المائى للبروتين عند درجة تركيز أيون الأيدروجين 5.5 ، 7.0 وذلك عند إضافة كلوريد الكالسيوم أو ثلاثى فوسفات الأدينوسين.

وكانت قيمة قوة الحفظ المائى للبروتين لعينات معاملة بخليط من كلوريد الكالسيوم مع ثلاثى فوسفات الأدينوسين أقل من العينات غير المعاملة وقد تحسنت قوة الحفظ المائى للبروتين لعينات معالجة بالأنزيمات، وذلك عند اضافة ثلاثى فوسفات الأدينوسين أو كلوريد الكالسيوم أو الاثنين معا.