

Biodegradation of exogenously applied silk peptides

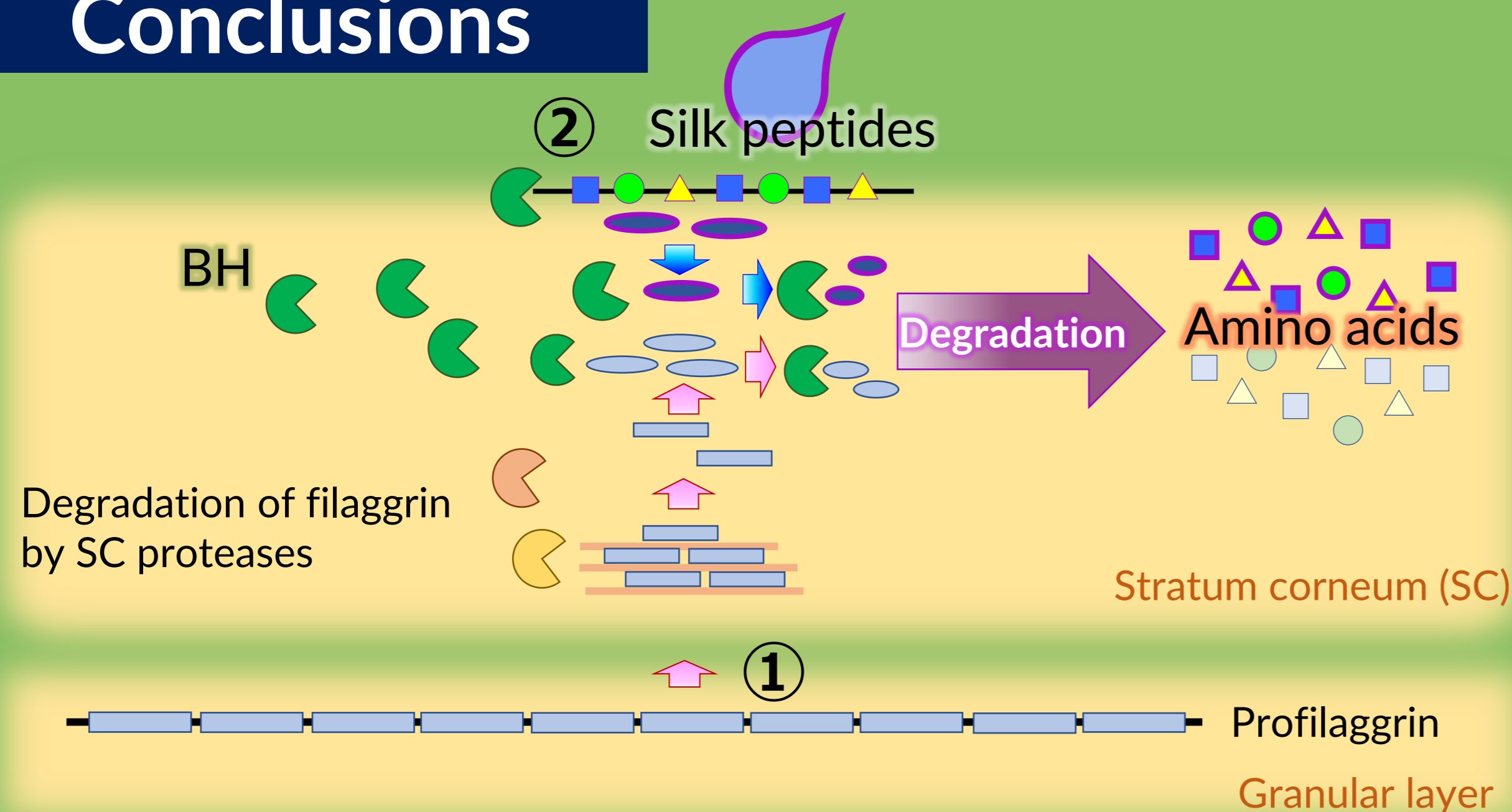
by stratum corneum cysteine proteases

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Conclusions



We found that exogenously applied silk peptides are degraded by cysteine proteases such as bleomycin hydrolase (BH), which is known to degrade filaggrin peptides into amino acids.

This suggests that amino acids of NMFs can be supplemented not only endogenously within the skin but also exogenously.

This concept of peptide degradation upon application to the SC provides a novel approach to skin moisturization!!

Introduction

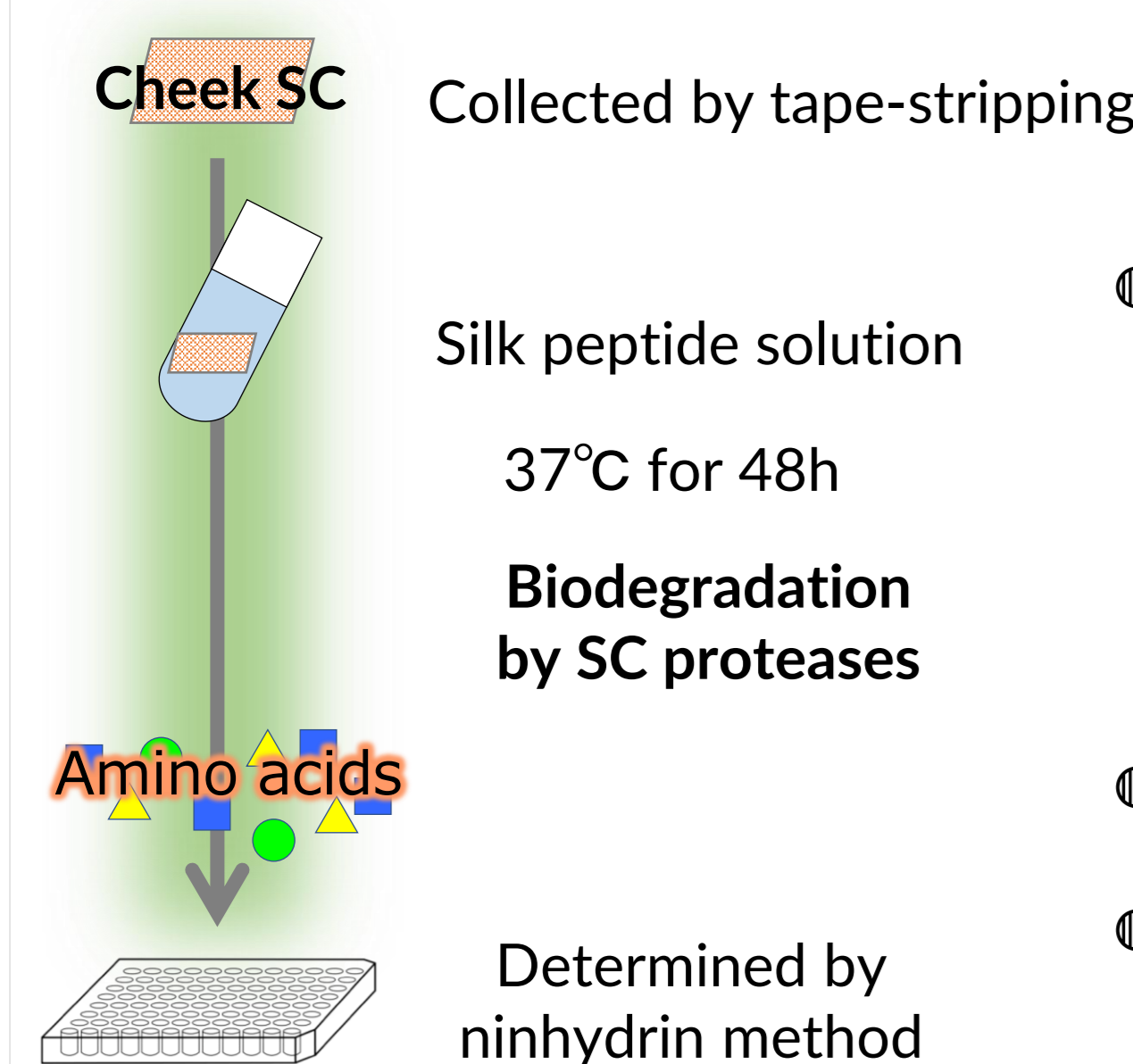
① "Conventional degradation route"

Previous studies revealed that filaggrin is degraded into amino acids by proteases such as BH and these amino acids may serve as NMFs.

② We hypothesized "New biodegradation route"

We aimed to know "whether exogenous peptides would be hydrolyzed to amino acids by SC proteases" and "what kind of proteases are involved?"

Materials & Methods

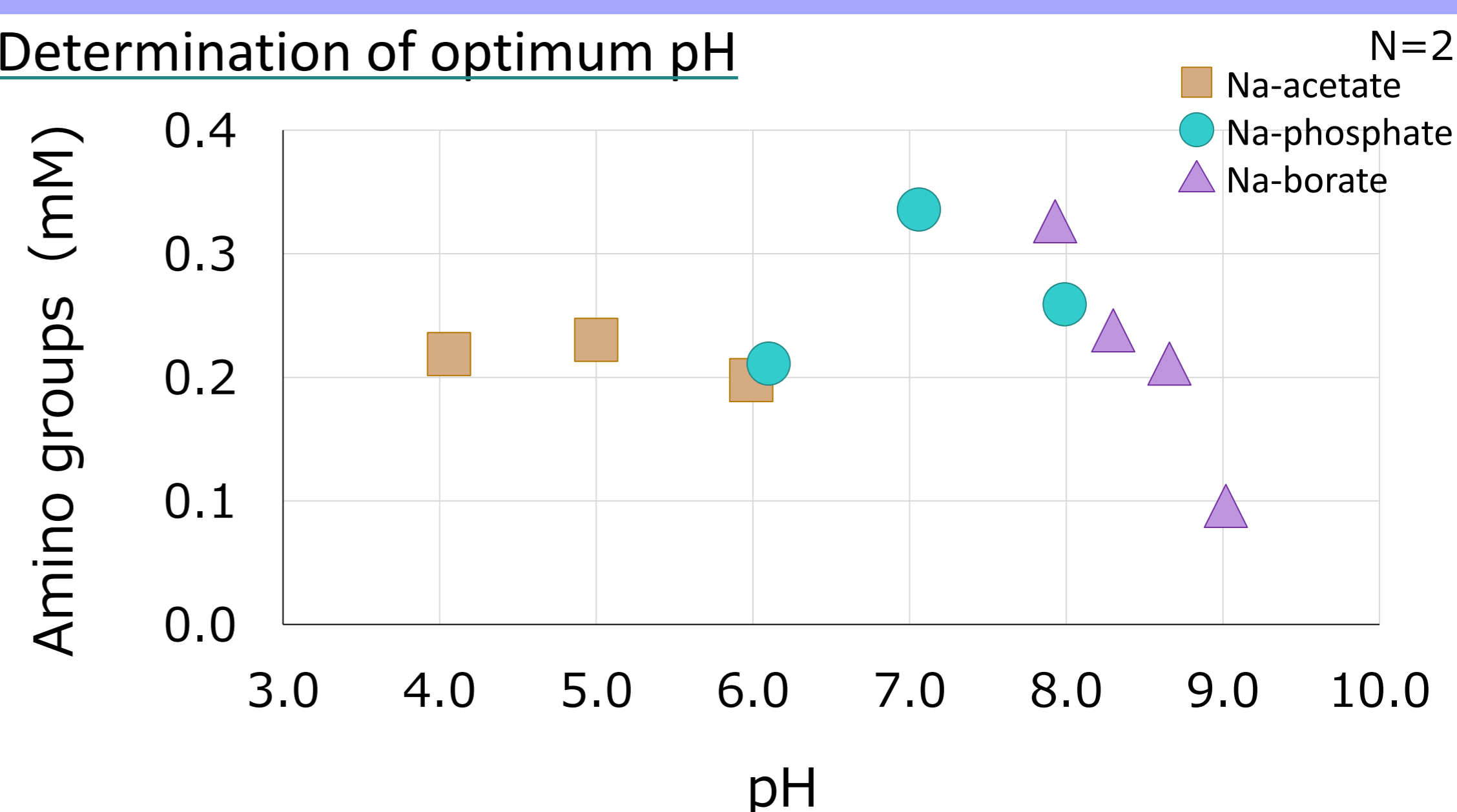


- Materials
 - Hydrolyzed silk
 - : Mixture of peptides (Average molecular weight 500 Da, Consists of high levels of Gly and Ala residues, Concentration 10%)
 - SILKPRO® F, IKEDA CORPORATION
- SC collection
 - Collected from the cheek by tape-stripping
- Monitoring of biodegradation of silk
 - The ninhydrin method,
 - MTP-900 Lab microplate reader (Corona Electric)

The protocol was approved by the ethics committee of Mukogawa Women's Univ.

Results & Discussion

Determination of optimum pH

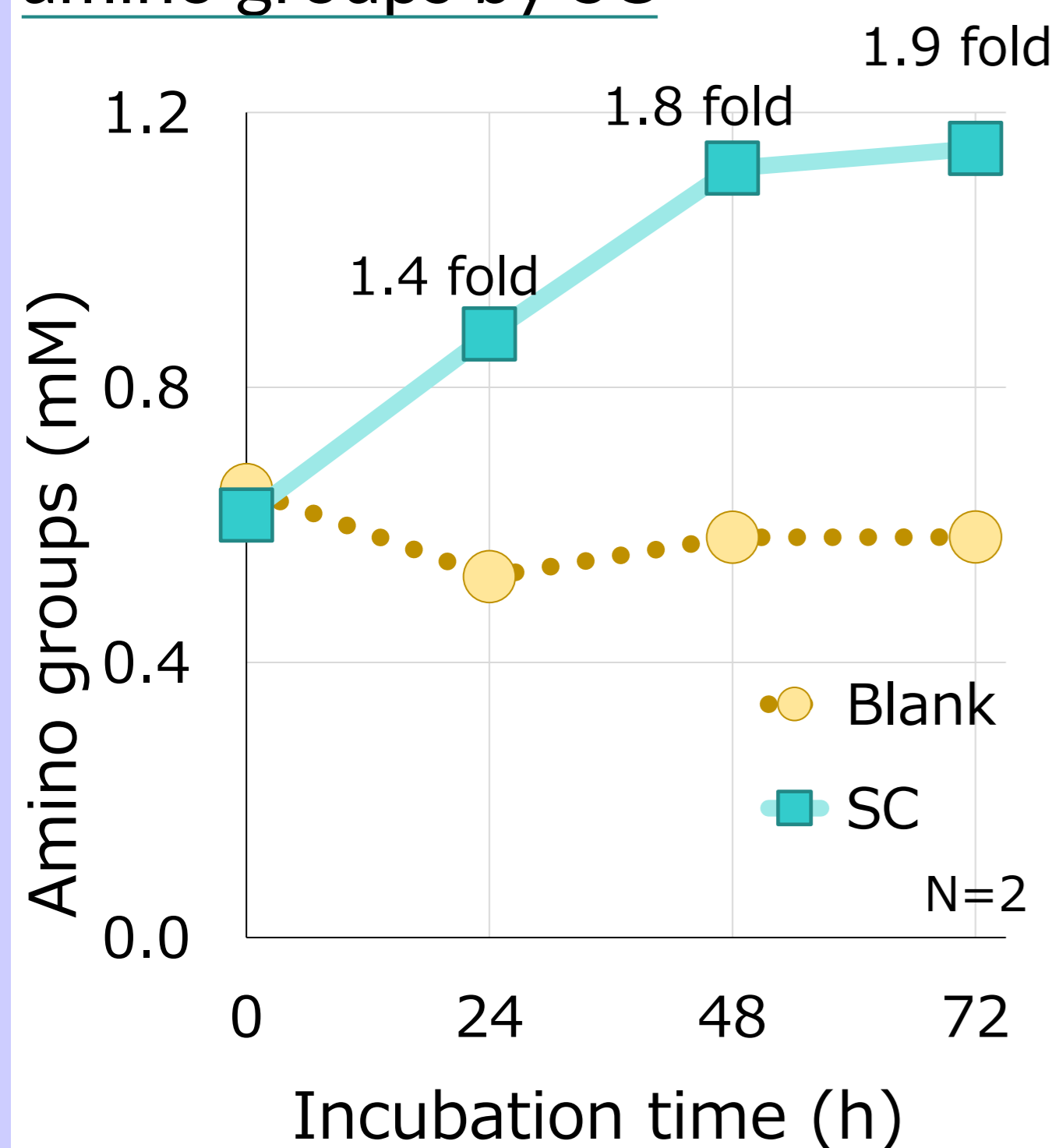


Effect of protease inhibitors and reducing agent

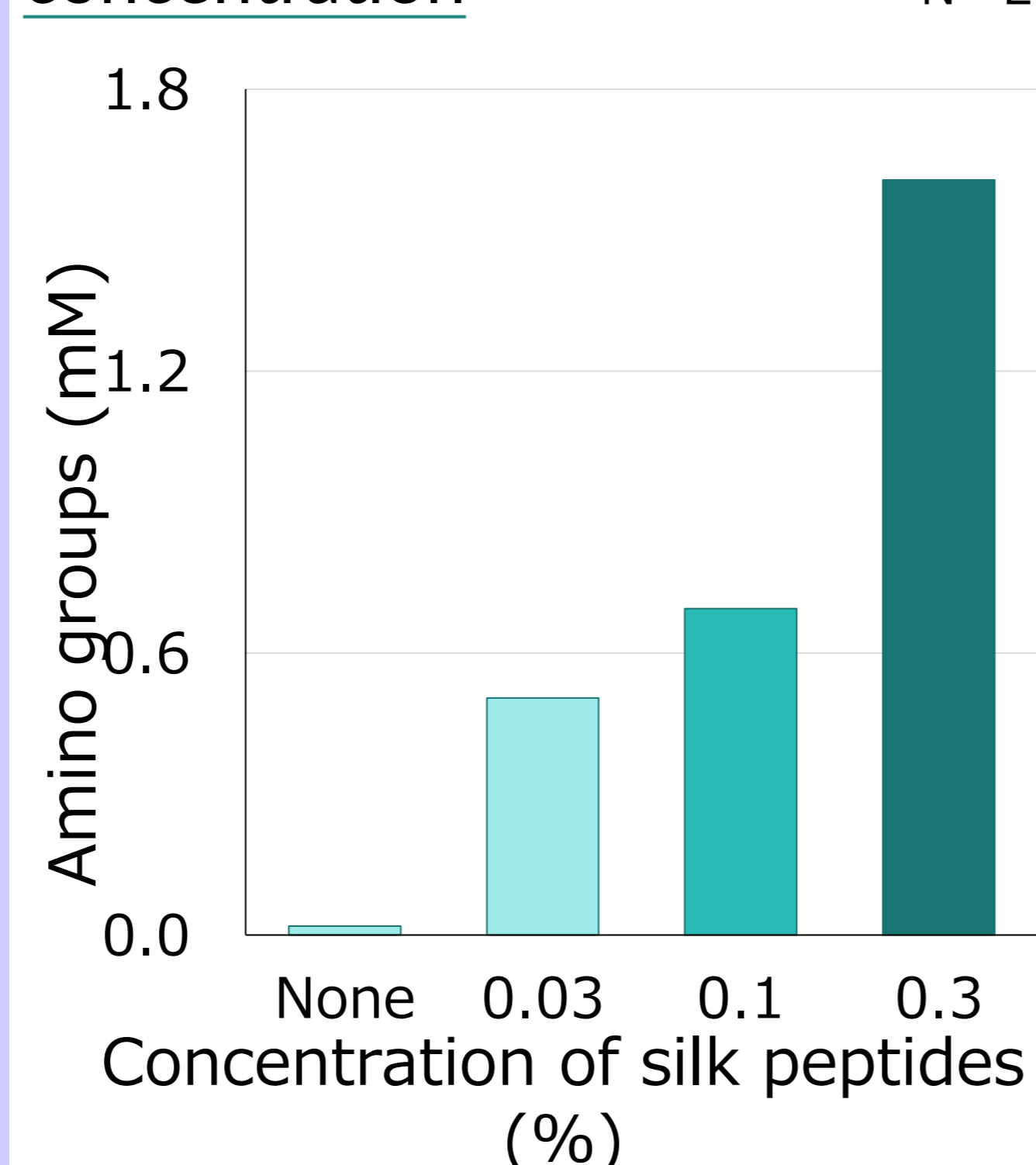
Inhibitor	Substances	Concentration	Relative activity (%)			
			Silk peptide degradation		Arg-MCA degradation	
			pH5.5	pH7.0	pH5.5	pH7.0
	None		100	100	100	100
	E-64	160 μM	40	23	30	14
	Leupeptin	120 μM	55	20	77	70
	Pepstatin	120 μM	97	81	43	112
	AEBSF	1 mM	112	132	81	92
	NEM	1 mM	75	72	45	15
	Iodoacetic acid	1 mM	16	50	24	19
	EDTA	1 mM	105	120	114	94
Reducing agent	DTT	10 mM	255	159	-	-

These results of silk peptide degradation showed the involvement of cysteine proteases, and is in agreement with the previous report [1].

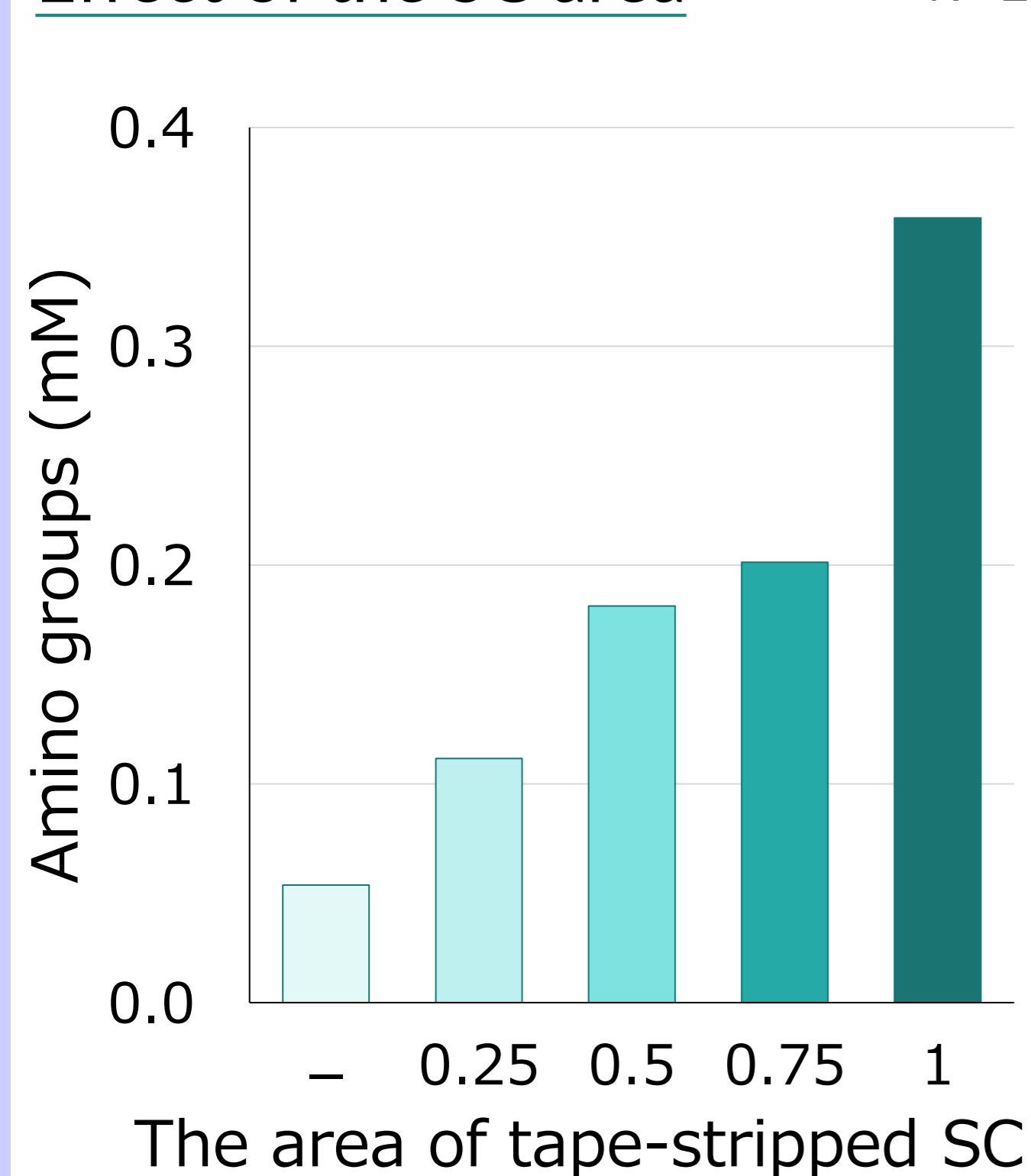
Time-dependent increase of amino groups by SC



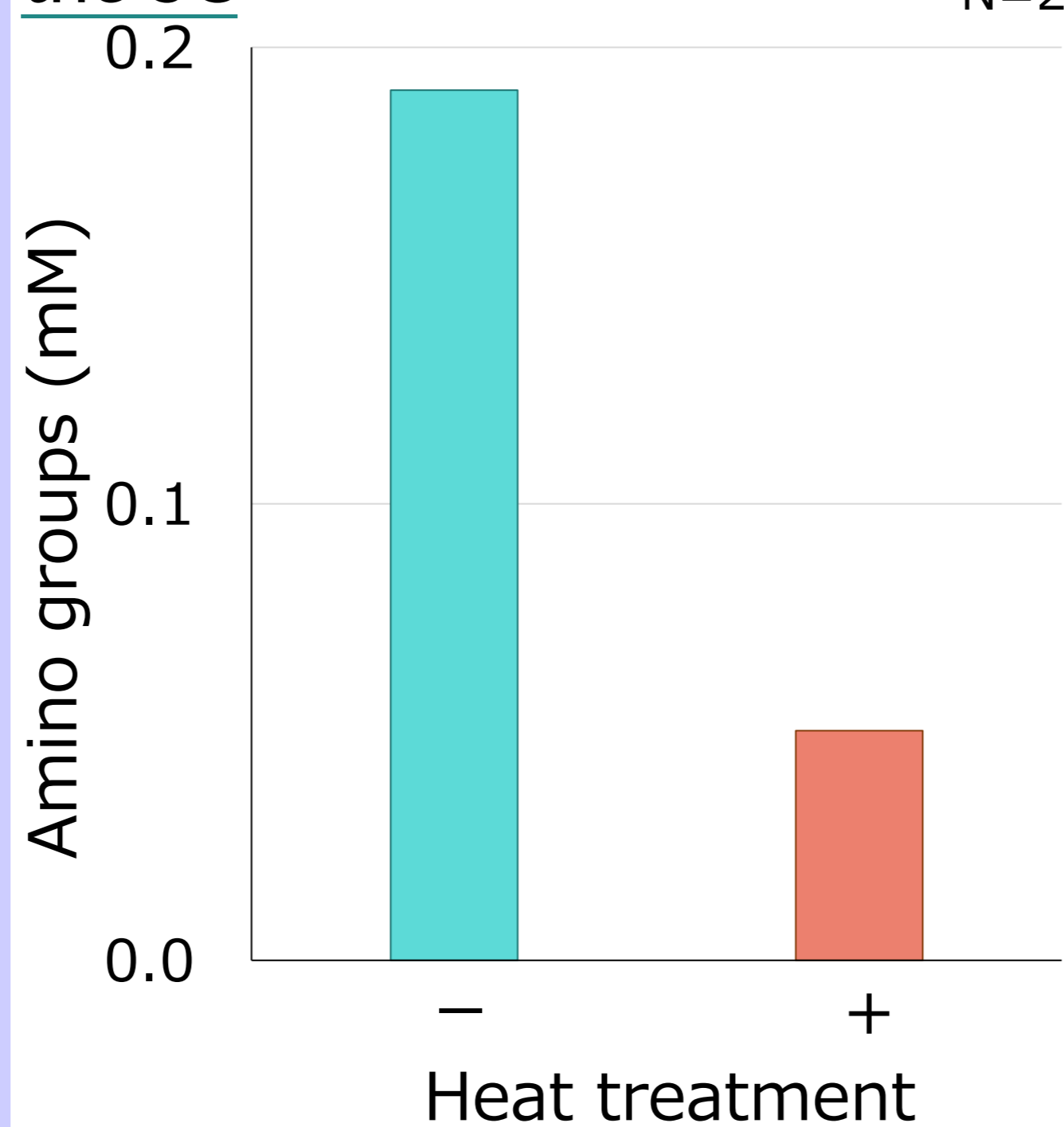
Effect of silk peptide concentration



Effect of the SC area

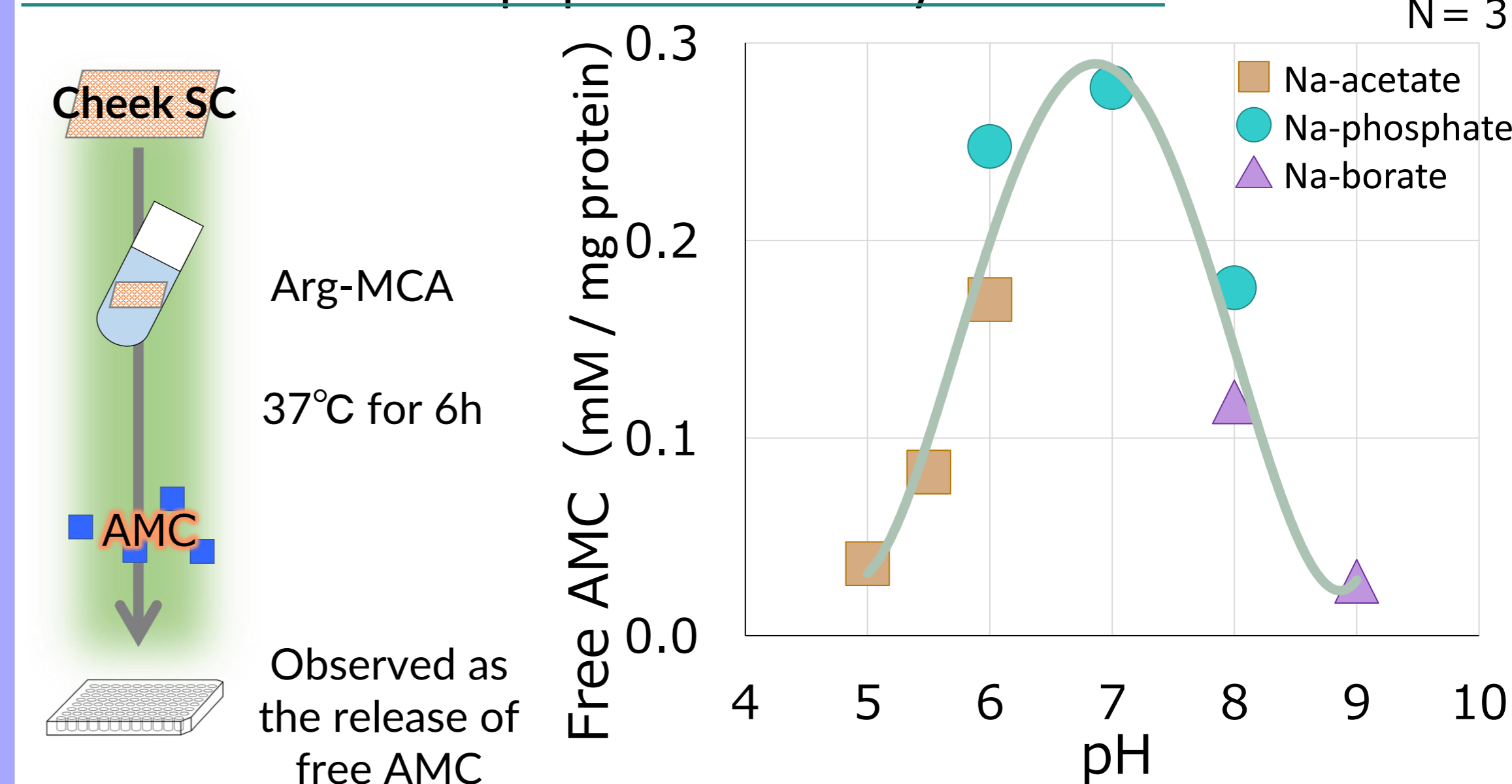


Effect of heat treatment of the SC



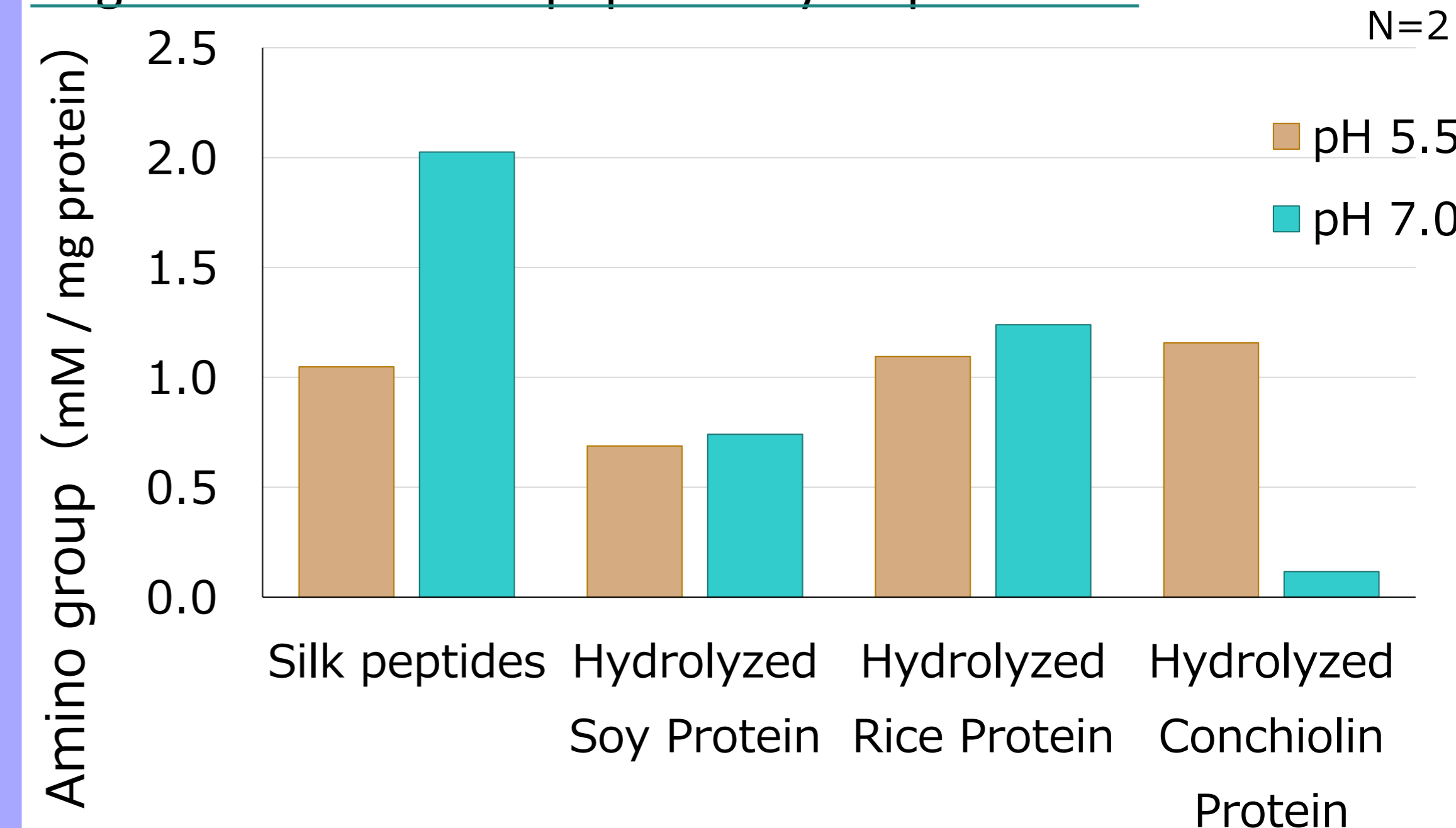
Biochemical characterization of silk peptide degradation by SC, including a time-course experiment, dependence on substrate dose, dependence on SC dose, and heat inactivation, clearly showed the involvement of certain proteases in the degradation.

Detection of aminopeptidase activity in the SC



The pH dependence of the aminopeptidase activity suggested that BH is involved in the degradation of peptide.

Degradation of other peptides by SC proteases



Each cosmetic ingredients were also degraded by SC proteases.

Reference

[1] Takeda A, Higuchi D, Yamamoto T, Nakamura Y, Masuda Y, Hirabayashi T, Nakaya K (1996) Purification and characterization of bleomycin hydrolase, which represents a new family of cysteine proteases, from rat skin. *J Biochem* 119:29-36.