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Kunio Honjo, Yoshinobu Tsukamoto, Ryo Nakamura, Akira Tsunemitsu and Toshiharu Matsumura

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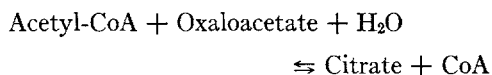
KUNIO HONJO, YOSHINOBU TSUKAMOTO, RYO NAKAMURA,
AKIRA TSUNEMITSU, and TOSHIHARU MATSUMURA

Department of Preventive Dentistry, Osaka University
Dental School, Osaka, Japan

SYNOPSIS IN INTERLINGUA

ACTIVITATE DE SYNTHASE DE CITRATO IN TISSU PERIODONTAL.—Con le utilisation del sensibile e convenibile essayo a fissura per ester thiolic, le activitate de synthase de citrato (EC 4.1.3.7; oxaloacetato-lyase de citrato) esseva determinate in le fractiones supernatante de gingiva, osso alveolar, e altere tissus de porco de India. Cortice de femore, osso alveolar, gingiva, hepate, e ren possedeva approximativamente 1/60, 1/33, 1/24, 1/12, e 1/4, respectivamente, le activitate medie per mg de extrahite proteina cardiac. Le activitate exprimate a base de unitates de peso de tissu humide esseva etiam le plus alte pro le corde. Illo de osso alveolar e de gingiva esseva approximativamente 1/440 e 1/40, respectivamente, del correspondent valor trovate pro le corde.

The citrate-condensing enzyme or citrate synthase (EC 4.1.3.7; citrate oxaloacetate-lyase) catalyzes the following reaction:



This enzyme is widely distributed in animal tissues and has been purified as a crystalline protein from pig heart by Ochoa, Stern, and Schneider¹ and Srere and Kosicki.² As to the activity of this enzyme in animal tissues, Dixon and Perkins³ have demonstrated that the citrate synthase activity of bone is relatively smaller than that of kidney and liver in rabbits. To our knowledge, no investigations have been made regarding the level of citrate synthase in periodontal tissues.

This communication describes a method for the determination of citrate synthase activity in gingiva and alveolar bone, and presents the results of assays carried out on normal guinea pigs. The enzyme activities in the heart, kidneys, liver, and femur cortex were also studied as comparisons.

Materials and Methods

PREPARATION OF TISSUES.—Male guinea pigs, 6 to 8 weeks old, and weighing 280 to 320 Gm., were used for this study. One hundred per cent (wet weight/volume) femur cortex, 33 per cent alveolar bone, 2 per cent gingiva, 0.5 per cent kidney (cortex of

kidney) and liver, and 0.25 per cent heart tissue extracts were prepared as described in previous reports.^{4, 5}

PREPARATION OF REAGENTS.—Acetyl-CoA* was prepared by the method of Simon and Shemin.⁶ Oxaloacetate* solution was freshly prepared and neutralized with basic Tris.†

ASSAY OF CITRATE SYNTHASE ACTIVITY.—Enzyme activity was determined by the method of Ochoa⁷ and Srere and Kosicki.² The principle of the method is based on the measurement of the change in absorbency at 233 m μ induced by both the cleavage of the thiol ester bound of acetyl-CoA and the utilization of oxaloacetate.

In a silica cuvette having a 1-cm. light path and 1-ml. capacity, incomplete reaction mixture was prepared as follows: 0.90 ml. of 0.1 M Tris-HCl buffer, pH 8.0, 0.01 ml. of approximately 0.008 M acetyl-CoA, and 0.04 ml. of tissue extract. After preincubation at 37° C. for 5 minutes, the final component, 0.05 ml. of 0.0076 M oxaloacetate, was added to the described reaction mixture. Readings of optical density at 233 m μ were taken at intervals of 30 seconds for 2 or 3 minutes. Enzyme analyses were performed with the spectrophotometer,‡ which was maintained at 37° C. by circulating water from a thermostat through thermo-

* Coenzyme A and oxaloacetic acid were obtained from Sigma Chemical Co., St. Louis, Mo.

† Tris(hydroxymethyl)aminomethane.

‡ Hitachi model EPU-2A, Hitachi, Ltd., Tokyo, Japan.

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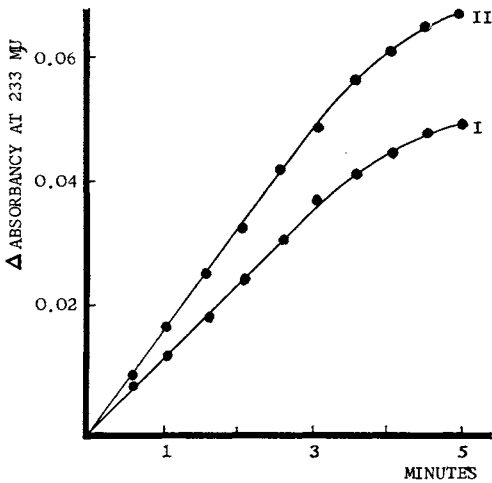


FIG. 1.—Time course of citrate synthase activity. I = gingiva; II = alveolar bone.

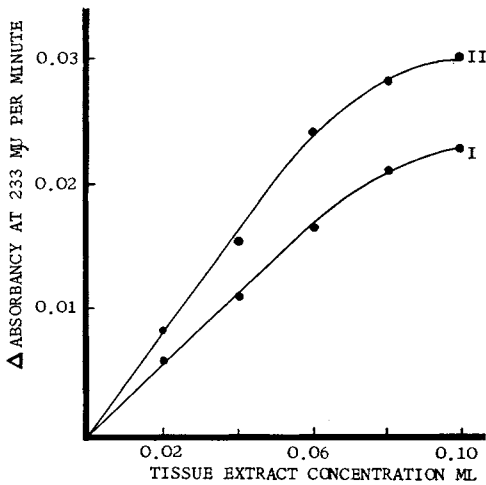


FIG. 2.—Relationship of enzyme activity to tissue extract concentration. I = gingiva; II = alveolar bone.

DEFINITION OF ENZYME ACTIVITY.—One unit of citrate synthase activity was defined as that amount of enzyme catalyzing the synthesis of 1 μM of citrate from acetyl-CoA and oxaloacetate/hour at 37° C. Enzyme activity was expressed both as units/gram of wet tissue and as units/mg. of extracted protein.

Results

The effects of time and tissue extract concentration on the enzyme activity were studied using the tissue extracts of gingiva and alveolar bone from normal guinea pigs. Figure 1 shows the course of enzymatic reaction that was obtained in the presence of tissue extracts and complete assay system. Although not shown in the Figure 1, when acetyl-CoA or oxaloacetate was omitted from the system, change in absorbancy was not observed. Figure 2 indicates the effect of tissue extract concentration on the enzyme activity with the dilute tissue extract. A straight line was followed up to 3 minutes in Figure 1 and to 0.06 ml. of tissue extract in Figure 2. The pH optimum of the enzyme was confirmed at 7.8–8.3 in every tissue. As a result of these studies, the final method for the determination of citrate synthase activity in crude tissue extracts was developed.

Data concerning citrate synthase activity of various tissues are given in Table 1. The highest activity, reported as units/mg. of extracted protein, was found in the heart. Femur cortex, alveolar bone, gingiva, liver, and kidney, each possessed approximately

TABLE 1
CITRATE SYNTHASE ACTIVITY IN
TISSUES OF GUINEA PIGS*

TISSUES	CITRATE SYNTHASE ACTIVITY	
	Per Gm. Wet Tissue	Per Mg. Extracted Protein
Femur cortex	2.2 ± 0.94	2.4 ± 0.72
Alveolar bone	12 ± 2.1	4.4 ± 0.77
Gingiva	132 ± 33	5.9 ± 1.51
Liver	973 ± 236	12.4 ± 2.46
Kidney	1,670 ± 376	38 ± 4.8
Heart	5,320 ± 770	144 ± 27.8

* Each value in the table is an average of individual determinations on 10 animals. The ± values are standard deviation of the means. Enzyme activity is expressed as μM of citrate formed/hour at 37° C.

spacers at both ends of the cell compartment. The decrease in absorbency at 233 mμ between 30 and 90 seconds after the start of the final reaction was used to calculate the enzyme activity. The formation of citrate was calculated assuming the combined molar absorbancy index of 5.4×10^3 .²

PROTEIN ESTIMATION.—The protein content of the tissue extracts was determined by the method of Lowry and others.³ Recrystallized egg albumin* was used for the standard protein solution.

* Nutritional Biochemicals Corp., Cleveland, Ohio.

1/60, 1/33, 1/24, 1/12, and 1/4.3 of the mean activity/mg. of extracted protein of heart, respectively. The heart gave also the highest activity, expressed as units/gram of wet tissue. Gingiva and alveolar bone each contained approximately 1/40 and 1/440 of the mean activity/Gm. of wet tissue of heart, respectively. The gingiva possessed about 10 times higher activity than the alveolar bone in units/Gm. of wet tissue, whereas the gingiva gave only 1.3 times greater activity than the alveolar bone in units/mg. of extracted protein.

Discussion

For the determination of citrate synthase activity, there are several methods, that is, colorimetric assay for citrate formation,^{1, 7} coupled malate dehydrogenase 340 m μ assay for the forward or back reaction,^{7, 9} and 233 m μ thiol ester cleavage assay.^{2, 7} Our investigation, using the sensitive and convenient thiol ester cleavage assay, demonstrated that the activity of citrate synthase can be measured in the gingiva and alveolar bone.

Because of the differences in methods and animals used, it is difficult to compare our data with those values of enzyme activities reported by Dixon and Perkins³ and Srere and Kosicki.² In activity/unit weight of wet tissue, the lowest activity was found in calcified tissues, but citrate synthase activity of alveolar bone was comparatively higher than that of femur cortex. The gingiva contained considerably greater activity than the alveolar bone in units/Gm. of wet tissue, whereas the activity of gingiva, expressed on the basis of unit content of extracted protein was slightly greater than that of alveolar bone. This may be the result of a differing content of extractable protein in each tissue.

Dickens¹⁰ has shown that 90 per cent or more of the citrate of the body was located in the skeleton. Dixon and Perkins³ have suggested that the higher citrate synthase and lower isocitrate dehydrogenase levels in bone regions may produce a locally increased concentration of citrate.

On the basis of our data, which have been previously reported by Honjo and others,⁵ it was found that the ratio of citrate synthase:isocitrate dehydrogenase (EC

1.1.1.42) of femur cortex, alveolar bone, gingiva, liver, kidney, and heart, each was approximately 0.2, 0.4, 0.6, 0.2, 0.4, and 1.1, respectively, in the activity expressed as units/mg. of extracted protein. From these results, it could not be confirmed that isocitrate dehydrogenase activity is relatively lower than citrate synthase in bone. Further research is necessary to investigate the mechanisms of citrate accumulation in calcified tissues.

Summary

A technic for measuring the enzyme activity of citrate synthase in the gingiva and alveolar bone of normal guinea pigs has been described. The enzyme activities in the heart, kidney, liver, and femur cortex were also determined as comparisons. When enzyme activity was expressed on the weight of the wet tissue, heart showed a high activity, kidney and liver intermediate activity, gingiva a relatively low value, and alveolar bone and femur cortex extremely low levels. The heart possessed also the highest activity expressed on the basis of content of extracted protein. The gingiva and alveolar bone each contained approximately 1/24 and 1/33 of the mean activity/unit content of extracted protein of heart, respectively.

References

- OCHOA, S., STERN, J. R., and SCHNEIDER, M. C. Enzymatic Synthesis of Citric Acid. II. Crystalline Condensing Enzyme, *J. biol. Chem.*, **193**:691-702, 1951.
- SRERE, P. A., and KOSICKI, G. W. The Purification of Citrate-Condensing Enzyme, *J. biol. Chem.*, **263**:2557-59, 1961.
- DIXON, T. F., and PERKINS, H. R. Citric Acid and Bone Metabolism. *Biochem. J.*, **52**:260-65, 1952.
- HONJO, K., TSUKAMOTO, Y., TSUNEMITSU, A., and MATSUMURA, T. Aconitase and Fumarase Activity in Periodontal Tissues, *J. dent. Res.*, **43**:495-500, 1964.
- HONJO, K., TSUKAMOTO, Y., NAKAMURA, R., TSUNEMITSU, A., and MATSUMURA, T. Isocitric Dehydrogenase Activity in the Gingiva and Alveolar Bone of the Guinea Pig, *Arch. oral Biol.*, **9**:699-704, 1964.
- SIMON, E. J., and SCHEMIN, D. The Preparation of S-Succinyl Coenzyme A, *J. Amer. chem. Soc.*, **75**:2520, 1953.
- OCHOA, S. *Biochemical Preparations*, vol. 5. D. SCHEMIN, editor. New York, John Wiley and Sons, Inc., 1957, p. 19-30.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. Protein Measurement with the Folin Phenol Reagent, *J. biol. Chem.*, **193**:265-75, 1951.
- STERN, J. R., SHAPIRO, B., STADTMAN, E. R., and OCHOA, S. Enzymatic Synthesis of Citric Acid. III. Reversibility and Mechanism, *J. biol. Chem.*, **193**:703-20, 1951.
- DICKENS, F. The Citric Acid Content of Animal Tissues, with Reference to Its Occurrence in Bone and Tumour, *Biochem. J.*, **35**:1011-23, 1941.