EFFECT OF CERTAIN PROTEINS AND AMINO ACIDS ON THE INCORPORATION OF ^{14}C-LEUCINE INTO THE FROG KIDNEY PROTEIN

A. SUBBARAMI REDDI AND KARUMURI S. SWAMI

Department of Zoology, Sri Venkateswara University, Tirupati

Summary : The effect of protein and amino acid charges was studied on protein synthesis, proteases and cytochrome oxidase in the kidney homogenates of frog. The gamma globulins and basic proteins, decreased the protein synthesis as well as the protein degradation while the albumins and acidic proteins showed an opposite effect, suggesting a high protein turnover rate in the kidney of frog. Lysine and glutamic acid followed the trend of gamma globulins and albumins, respectively. The energy producing systems as indicated by cytochrome oxidase activity was proportionately stepped up by the albumins. It is suggested that the elevation of albumin content in the kidney may promote the turnover rate of protein and this information may be helpful in understanding the mechanism of kidney degeneracy.

Key Words : *protein synthesis role of protein charges in protein synthesis protein turn-over rate kidney proteins.*

INTRODUCTION

The pathologic kidney is known to lose its efficiency in selective excretion of water, salts and urea and in some cases results in albuminurea. The kindney tissue undergoes disintegration presumably owing to high protein catabolism suggesting the possibility of increased proteolysis. Since enzyme distrubances lead to pathologic states of tissues and a proper regulation restores normal metabolism, a precise understanding of the mechanism of enzyme catalyzed reactions may help in correcting tissue disturbances. Previous studies on the gastrocnemius muscle (4, 6) and kidney (11, 12) of frog and sheep brain (9) indicated that the enzyme velocities in heterogeneous systems are under the influence of the charge density contributed by proteins and amino acids in the environment. It has been suggested that a protein environment rich in positive charges elevated the activity levels of lactate, succinate and glutamate dehydrogenases and decreased those of proteases (4, 6). The protein and amino acid environments relatively rich in negative charges induced an opposite effect. Thus the environmental charges seem to play an important role in the regulation of enzyme activity either by activating or inhibiting the enzyme systems. In the present investigation an attempt was made to study the protein metabolism in the kidney of frog as regulated by exogenously added protein and amino acid charges.

MATERIALS AND METHODS

The CNS of *Rana hexadactyla* was destroyed by pithing and the kidneys were quickly excised. They were washed in amphibian Ringer's medium (2) and were allowed to stand for

20 min to recover from shock effects. The Ringer's medium was aerated to provide normal carbon dioxide tension. The peritoneum and the adrenal body were removed and the blood was blotted out with a filter paper.

Protein fractionation : 10 to 12 kidneys were pooled and a 25% homogenate (wt/vol) was prepared in 0.25 M sucrose and centrifuged at 2000 *g* for 15 min. The albumins and gamma globulins were extracted as per the method of Cohn *et al.* (3).

Acidic and basic proteins : A 25% homogenate (wt./vol.) of pooled up kidneys was prepared in 0.25 M sucrose and centrifuged at 2000 *g* for 15 min. The sediment was separated from the supernatant and the latter was divided into two fractions of 10 *ml* each. To each fraction was added 5 *ml* of 0.1 M acetate buffer of pH 5.0 and to the other 5 *ml* of 0.1 M phosphate buffer of pH 8.0. The proteins were extracted in cold for 6 hrs and centrifuged. The sediments were dissolved separately in 5 *ml* of 0.25 M sucrose solution. The proteins obtained at pH 5.0 and pH 8.0 are termed acidic and basic proteins, respectively. The protein content in all of the fractions was determined with the biuret method as modified by Palladin (8) and the levels were equalized by appropriate dilutions with sucrose that each *ml* contained 1 *mg* protein. To all of the experimental reaction mixtures was added 0.25 *ml* of this solution containing 0.25 *mg* of protein.

Incorporation of leucine-1-^{14}C by the kidney proteins : The incorporation of labeled leucine-1-^{14}C (1 μC with sp. activity 5 x 0.1 mC) into the kidney proteins was studied using the method of Wang and Patel (14) in kidney homogenates enriched with 0.25 *mg* of isolated renal protein or commercial gamma globulins (Nutrition Biochemical Corporation, Cleveland, Ohio) or bovine albumin (L. Light and Co. Ltd., Colnbrook, England) or glutamic acid (B.D.H., India) or lysine (B.D.H., India). The radioactivity was measured with G.M. Counter (Atomic Energy Supply, Type 1-1030/1150 2-1350 V ; Trombay, Bombay). The incorporation of label into the kidney proteins was expressed as specific activity in terms of counts/min/mg protein. The protein content was estimated by the biuret method.

Protease activity : A 2% kidney homogenate (wt./vol.) was prepared in 0.25 M sucrose and centrifuged at 2000 *g* for 15 min. To 1.0 *ml* of 0.1 M phosphate buffer (pH 4.5) was added 1.0 *ml* of supernatant followed by 1.0 *ml* of casein prepared in 0.25 M NaOH. This formed the control. The experimental samples received 0.25 *mg* of isolated renal protein or commercial gamma globulins or bovine albumin. The control and experimental reaction mixtures were incubated for 4 hrs. at 37°C and then the reaction was arrested by the addition of 2.0 *ml* of 10% TCA. After centrifugation at 1500 *g* for 15 min the free amino acid content was determined by the ninhydrin method as given in Snell and Snell (10) in the supernatant. Control experiments in which the homogenate was deleted from the reaction mixture showed negligible contribution of amino acids by alkaline hydrolysis of casein that might be brought about by the experimental conditions employed.

Cytochrome oxidase assay : The level of cytochrome oxidase was determined by the

TABLE I: *Levels of protein synthesis expressed as specific activity in terms of counts/min/mg protein, protease activity expressed as μg of tyrosine units/gm/hr and cytochrome oxidase activity expressed as μg of diformazan/gm/hr. Each value represents an average of ten individual experiments with its standard deviation. S means significant; NS means non-significant*

	Control	*Commercial gamma globulins*	*Commercial albumins*	*Acidic proteins*	*Basic proteins*	*Isolated gamma globulins*	*Isolated albumins*	*Glutamic acid*	*Lysine*
Protein Synthesis	107.27 ± 12.74	89.60 ± 17.64 S	123.00 ± 21.23 NS	126.99 ± 11.85 S	92.10 ± 12.01 S	84.06 ± 6.45 S	134.04 ± 5.67 S	124.78 ± 14.27 S	84.75 ± 24.33 S
Protease activity	780.00 ± 97.97	647.00 ± 41.90 S	1320.00 ± 203.90 S	980.00 ± 74.84 S	66.00 ± 80.00 NS	686.00 ± 38.26 NS	1160.00 ± 102.00 S	1075.00 ± 80.90 S	705.00 ± 45.35 NS
Cytochrome oxidase.	430.00 ± 43.00	584.00 ± 55.44 S	755.00 ± 60.00 S	865.00 ± 64.42 S	690.00 ± 68.20 S	710.00 ± 64.42 S	890.00 ± 81.53 S	725.00 ± 58.20 S	645.00 ± 48.55 S

colorimetric method of Oda *et al.* (7) in the kidney homogenates enriched with the protein as given above.

All of the experimental procedures were carried out in cold (4°C) and the observations were subjected to statistical validity by calculating Student's significance test.

RESULTS

The amino acid label studies showed 21, 16 and 14 per cent decrease of incorporation into the kidney protein in homogenates enriched with the gamma globulins, both isolated and commercial proteins, and basic proteins, respectively. The kidney homogenates with the isolated and commercial albumins and acidic proteins elevated the incorporation of label by 23, 15 and 18 per cent, respectively, as compared to the control, the enhancement by the commercial albumins being non-significant. Glutamic acid showed 16 per cent increase, while lysine showed 20 per cent decrease on the incorporation of label into the kidney proteins.

The proteolytic activity decreased in kidney homogenates enriched with the commercial as well as isolated gamma globulins, while it increased in homogenates containing commercial, isolated albumins and acidic proteins. Though the isolated gamma globulins and basic proteins inhibited the enzyme activity by 12 and 15 per cent, respectively, their inhibition was not significant. Between the albumins and acidic proteins, the activation was more with the former than with the latter. Glutamic acid stimulated proteolytic activity, while lysine inhibited it, the inhibition being non-significant.

All the proteins, in general, elevated the level of cytochrome oxidase activity significantly. Among them, the activation was more in homogenates with the isolated albumins followed by the acidic proteins and commercial albumins. The elevation by the gamma globulins and basic proteins was more or less equal with corresponding lesser activation by the commercial gamma globulins. Both amino acids significantly elevated the levels of cytochrome oxidase activity.

DISCUSSION

The rate of protein synthesis, in terms of the incorporation of leucine-1-^{14}C into the proteins of the kidney, was elevated in the homogenates enriched with albumins and acidic proteins, while the gamma globulins and basic proteins had decreased the incorporation. Since the major protein component in the amphibian kidney is gamma globulin (13), it is likely that the albumins stimulated the gamma globulin synthesis, while gamma globulins inhibited which is probably a product inhibition.

Tissues involved in elaborate protein synthesis are known to have a high level of proteolytic activity (1). In order to maintain nitrogen balance, the proteolytic activity should step up in tissues not involved in growth. Similar relationship has been envisaged in the present study. The level of proteolytic activity increased in the kidney homogenate containing albumins and acidic proteins, while the gamma globulins and basic proteins decreased the level of acti-

vity. Thus, the exogenously added proteins induced an effect similar to that of protein synthetic potential. An increased level of albumins elevated protein synthesis as well as protein degradation suggesting a high turnover of proteins in the kidney. The elevated turnover of proteins by the albumins is expected to be associated with an elevated synthesis of messenger RNA and the increased synthesis of messenger RNA should be associated with an elevated synthetic activity of DNA. Gamma globulins produced an opposite effect.

Since protein synthesis is an energy requiring process, an increase in protein synthetic potential should require an increased energy supply. In the present study, the level of activity of cytochrome oxidase was considered an index of the energy yielding oxido-reduction systems associated with mitochondria. Enrichment of kidney homogenates with the albumins and acidic proteins increased the protein turnover and also the level of activity of cytochrome oxidase, while gamma globulins brought out lesser elevation of activity. Hence, the energy producing system is also stepped up proportionately by the albumins.

In pathologic states, the kidney undergoes degeneration and loss of kidney material involving proteins loss. The incompatible protein metabolism could be due to disturbances in protein turnover. Since exchange of protein between plasma and tissues is known (5) an elevation of plasma albumin may result in the elevation of the albumin content in the kidney and it may bring about an efficient protein turnover and it is likely that the protein disturbance in kidney could be corrected. As the plasma albumin level is dependent on the tissue activity, it is likely that stepping up of the liver activity could correct kidney degeneracy.

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