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DEAMINATION OF AMINO ACIDS BY 21-DEHYDROPREDNISOLONE

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SUMMARY

The reaction of 11β, 17α-dihydroxy-1,4-pregnadiene-3,20-dion-21-al (21-dehydroprednisolone) with amino acids was investigated. The rate of reaction as measured by the amount of keto acids formed was most rapid with sodium glutamate. Evidence is presented suggesting that the free γ-carboxylic group of glutamic acid influences the rate of deamination.

INTRODUCTION

THE reaction of 21-dehydrocorticosteroids with amino acids results in the formation of labile steroid–amino acid complexes (Schiff bases) which degrade to 21-aminocorticosteroid and keto acids[1, 2]. The 21-aminocorticosteroid reacts with excess 21-dehydrocorticosteroid in the presence of phosphate ions to yield a yellow disteroidamine complex[1, 2]. In the present study, the rate of deamination of amino acids by 21-dehydroprednisolone (11β,17α-dihydroxy-1,4-pregnadiene-3,20-dion-21-al) (21-DHP) was measured to determine the experimental conditions which influence the reaction.

EXPERIMENTAL

The material and methods were as described in previous reports[1, 2]. Prednisolone (11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione) was purchased from Nakarai Chemical Co., Osaka, Japan, and converted to 21-DHP by oxidation with methanolic copper acetate according to a modified method of Lewbart and Mattox[3]. Amino acids were purchased from Takara Kohsan Co., Ltd., Tokyo, Japan.

21-DHP was dissolved in ethanol (5·5 mM) except for the experiment shown in Fig. 3, wherein a concentration of 1·8 mM was used. A mixture containing 1·5 ml of 10 mM amino acid solution, 1·5 ml of steroid solution and 0·3 ml of a 0·2 M phosphate buffer (pH 7·4) or water was prepared and incubated at 37°C for 240 min.

Keto acids were determined by extracting the reaction mixture containing 21-DHP and amino acid with dichloromethane to remove the free steroids and Schiff bases. To the aqueous phase, 0·1 vol. of 0·2% 2,4-dinitrophenylhydrazine in 2 N HCl was added and the mixture incubated at 37°C for 20 min. The mixture was extracted with ethyl acetate until the yellow product was completely removed. The ethyl acetate fraction was extracted several times with 10% Na_2CO_3 solution and the aqueous fractions were pooled. To 4 ml of the Na_2CO_3 solution 0·8 ml of 7 N NaOH was added. Within 2–3 min after color development, absorbance was measured at 500 or 460 nm.

The reaction mixture was extracted with dichloromethane under neutral (pH 7·4) and alkaline (pH 9–10) conditions and amide-N-determined. The

aqueous phase was digested in 1·5 N sulfuric acid at 120°C for 60 min. Nitrogen content of the digested solution was estimated with Nessler's reagent.

RESULTS

Figures 1 and 2 show the amount of α-keto acid formed following the interaction of 21-DHP with various amino acids in phosphate buffer at neutral pH or in a medium without phosphate ions. Relatively large amounts of keto acids were formed with glycine, histidine, hydroxy and sulfhydryl amino acids when the reactions were carried out in phosphate buffer (Fig. 1). Of the amino acids tested, glutamic acid yielded the greatest amount of keto acid. The amount of α-ketoglutarate formed was about 6 times greater when the reaction was carried out in a medium without phosphate ions than in phosphate buffer. Figure 3 shows the time course of α-ketoglutarate formation during the reaction of 21-DHP with glutamic acid. The amount formed increased with time and was maximal after 18–20 hr of incubation. To determine whether or not amino acids with similar structure were rapidly deaminated, aspartic acid and glutamine were incubated with 21-DHP (Fig. 4). A rapid formation of keto acid was observed only with glutamic acid. The amount of disteroidamine in the reaction mixture can be estimated by measuring the absorbance at 435 nm[1, 2]. The absorbance value of the reaction mixture of 21-DHP and glutamine was greater than that with glutamic acid (Fig. 5). That 21-DHP may react with the amide-N of glutamine is suggested

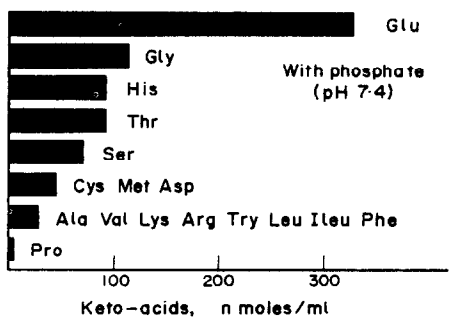

Fig. 1. Amount of keto acids formed from the reaction of 21-dehydroprednisolone with various amino acids in phosphate buffer. The reaction mixtures were incubated at 37°C for 240 min.

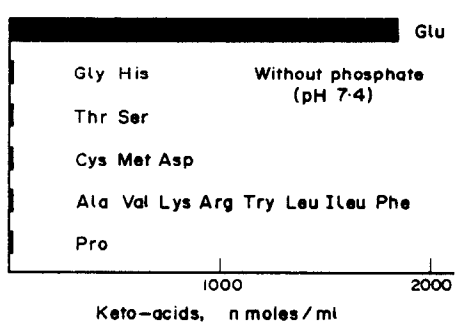

Fig. 2. Amount of keto acids formed from the reaction of 21-dehydroprednisolone with various amino acids in the absence of phosphate ions. The reaction mixtures were incubated at 37°C for 240 min.

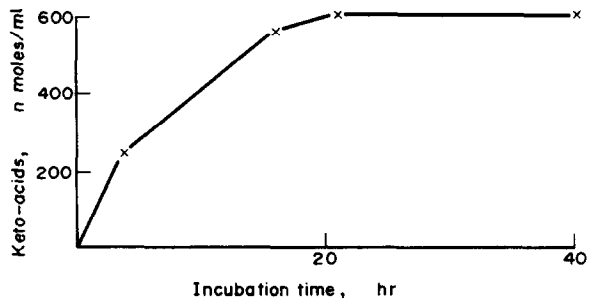

Fig. 3. Time course of α-ketoglutarate formation following the reaction of 21-dehydroprednisolone with glutamic acid. The mixtures were incubated at 37°C.

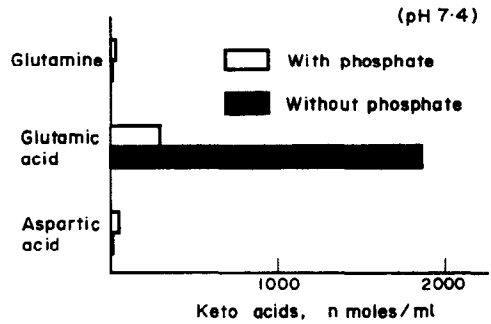

Fig. 4. Amount of keto acids formed during the reaction of 21-dehydroprednisolone with glutamine, glutamic acid or aspartic acid. The reaction mixtures were incubated at 37°C for 240 min.

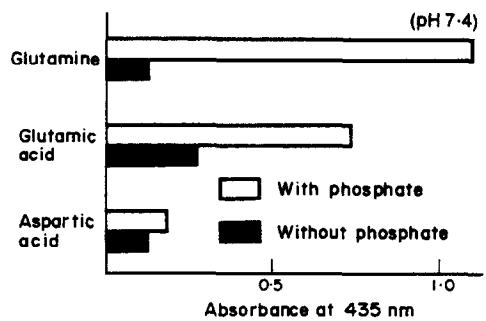

Fig. 5. Changes in absorbance following the reaction of 21-dehydroprednisolone with glutamine, glutamic acid or aspartic acid. Absorbance of the reaction mixture was measured after incubation at 37°C for 240 min.

by the finding that the amount of amide-N in the reaction mixture following the incubation of 21-DHP with glutamine in phosphate buffer decreased with time (1·2 μmol/240 min). The effect of N-acetylglutamic acid on the amount of keto acid formation from the reaction of alanine and 21-DHP was studied (Table 1) to establish whether or not the γ-carboxylic group of glutamic acid may influence the rate of deamination of amino acids by intermolecular interaction. N-acetylglutamic acid did not affect the reaction.

Table 1. Effect of N-acetylglutamate on the deamination of alanine by 21-dehydroprednisolone

Tube No.	0·1 M Alanine (ml)	0·1 M N-Acetylglutamate (ml)	5·5 mM 21-DHP (ml)	0·2 M Phosphate Buffer (ml)	H_2O (ml)	α-Keto Acid Formed (nmol/ml/240 min)
A	0·75	0·75	1·5	0·3	—	64
B	0·75	—	1·5	0·3	0·75	63·5
C	0·75	0·75	1·5	—	0·3	6·5
D	0·75	—	1·5	—	1·05	6·0
E	—	0·75	1·5	0·3	0·75	0

All tubes were incubated at 37°C for 240 min.

DISCUSSION

The results of the present study suggest that the Schiff base complex of 21-DHP and glutamic acid was labile and degraded to α-ketoglutarate and 21-aminoprednisolone. The lability of the steroid-glutamate complex may be dependent on the reactivity of the γ-carboxylic group. This notion is supported by the observation that only a small amount of keto acid was formed when 21-DHP was incubated with glutamine or aspartic acid. The rapid deamination may be due to an intramolecular interaction since N-acetyl-glutamic acid did not affect the rate of deamination of alanine by 21-DHP. An intramolecular cyclic rearrangement may be the basis for the reaction since di- and tripeptides are rapidly deaminated by 21-DHP[2].

The finding that the amount of keto acid formed from the reaction of 21-DHP with glutamate was significantly lower when carried out in phosphate buffer than in a medium without phosphate ions (Figs. 1 and 2) is at variance with the results obtained with other amino acids[2]. This paradoxical effect with glutamate is unexplained. However, it appears that phosphate ions may influence the formation of disteroidamine[1, 2], which would affect the levels of 21-DHP and α-ketoglutarate and the rate of reaction.

The results of the present finding may be applicable to the findings of Feigelson and Feigelson[4] that glutamic acid administered parenterally to adrenalectomized rats mimics cortisone action. The influence of cortisol on glutamic acid metabolism under physiological conditions is not known. Furthermore, neither 21-dehydrocortisol nor 21-aminocortisol have been demonstrated to exist in mammalian tissues.

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