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INTERACTIONS OF ESTRADIOL-17β WITH AMINO ACIDS

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SUMMARY

The solubility of estradiol-17β in phosphate buffer and in solutions containing phosphate buffer and amino acids was estimated. Arginine, aspartic acid, glutamic acid, lysine, tryptophan and tyrosine significantly increase the solubility of estradiol-17β. A slight increase in solubility of estradiol-17β was observed in solutions of proline and histidine, while other amino acids had no effect.

INTRODUCTION

In 1960, ABELSON *et al.*[1] reported on complex formations between testosterone and certain amino acids in buffered solutions. Of the 18 amino acids tested tryptophan, tyrosine, phenylalanine, histidine, arginine and lysine significantly increased the solubility of testosterone over that observed in buffer alone at the corresponding ionic strength.

In view of the renewed interest in steroid–protein interactions as a basis for assay procedures[2] and the mechanism of steroid hormone action[3] a study of possible associations between estradiol-17β and amino acids seemed desirable as an initial step in elucidating the nature of steroid–protein interactions. As has been pointed out already[1] such association would account for only part of the interactions between proteins and estradiol-17β.

In the present communication the influence of amino acids on the solubility of estradiol-17β in phosphate buffer is reported. Like all thermodynamic parameters solubilities are defined only at equilibrium. Information as to whether equilibrium has been reached can be obtained by estimating solubilities from both undersaturation and supersaturation. It is evident that the data presented here were obtained exclusively by reaching the equilibrium from undersaturation. However, it was shown that the solubilities were independent of the time of incubation. In view of previous work on related topics[1, 6, 7] this approach was thought to give adequate information.

MATERIALS AND METHODS

Estradiol-17β was a gift from Organon Laboratories, Ltd., Morden, Surrey, England. No impurities could be detected by thin-layer chromatography. The Kober reaction for estradiol was performed as described[4]. In this reaction the corrected optical density for 10 μg was $0{\cdot}666 \pm 0{\cdot}006$ ($n = 20$). The identity of the estradiol-17β was confirmed by I.R. spectroscopy (KBr-pellet).

Buffers: potassium dihydrogen phosphate–disodium hydrogen phosphate buffer pH 7·2 (25°), ionic strength 0·05, 0·1 and 0·2 respectively were prepared[5]. At 37° a pH of 7·17 was measured.

Amino acid solutions in phosphate buffer, ionic strength 0·10, were prepared.

Equilibration of excess estradiol-17β with sodium chloride solutions, phosphate buffer, or amino acid solutions in phosphate buffer: In a test tube 1 ml of an ethanolic estradiol-17β solution (1 mg/1 ml) was evaporated to dryness in a stream of nitrogen. To the dry residue 10 ml of sodium chloride solution (0·02 M, 0·05 M, 0·1 M, 0·2 M, 0·4 M, 0·6 M), or phosphate buffer (ionic strength 0·05, 0·1 or 0·2), or amino acid in phosphate buffer (ionic strength 0·1) respectively, was added. The tube was stoppered and incubated at 37° in a water bath until equilibrium was reached (20–24 hr). The solution was shaken at regular intervals. At the end of the incubation it was filtered through sintered glass (G-4). New (i.e. not previously used) AG filters porosity 4 were used. The manufacturers' specifications are: maximum pore diameter within the range 5–10 μ. The filtrate was extracted once with an equal volume of freshly distilled diethylether. The volume of the ether extract was adjusted to 10 ml. Aliquots of the ether extracts were

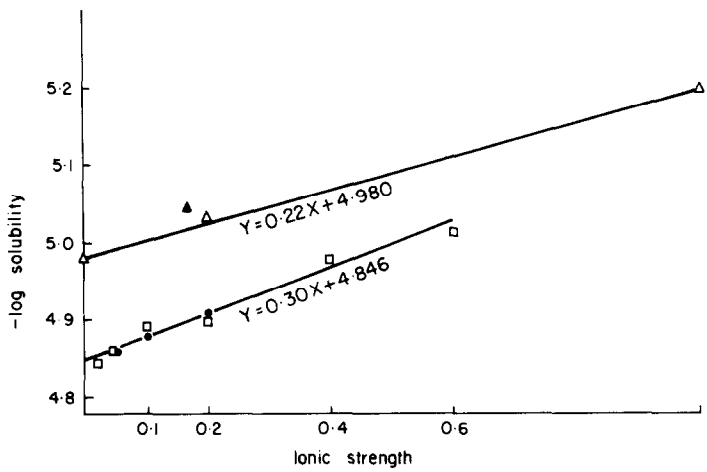

Fig. 1.

evaporated to dryness in a stream of nitrogen and used for the quantitative estimation of estradiol-17β by the Kober reaction[4]. No increase in the solubility of estradiol-17β was observed when increasing amounts were incubated with a constant volume of buffer. No increase in the solubility of estradiol-17β was found when the incubation time was increased to 48 hr or 72 hr. Losses of steroids in the glass filter were shown to be insignificant: when one of two sets of six samples each of estradiol-17β in the phosphate buffer was centrifuged for the separation of excess solid from dissolved estradiol-17β, rather than filtered as described, the difference of the mean (3·5 per cent) of the two sets was not significant. It is not impossible that the amino acids might influence the amount of steroid retained in the filter but no further tests in this regard were done.

RESULTS AND DISCUSSION

The results of the estimations of estradiol-17β in the ether extracts are shown in Table 1. If the negative logarithm of the solubility in buffer and sodium chloride solutions are plotted over the ionic strength, a linear relationship is found: $pS = pS_0 + K(\Gamma/2)$, in which pS is the negative logarithm of the solubility in moles per

Table 1. Solubility of estradiol-17β in phosphate buffer of 0·10 ionic strength in the presence of amino acids. "Mean" is the average of the mean solubilities of estradiol-17β in amino acid solutions that do not significantly influence its solubility

Amino acid	Molarity	Number of tests	Estradiol-17β moles $\times 10^{-6}$ per liter
(1) No significant effect on solubility of estradiol-17β			
L-Cysteine Hydrochloride	0·020	8	12·5 ± 1·1
DL-Phenylalanine	0·050	6	13·3 ± 0·8
DL-Serine	0·050	6	13·1 ± 2·1
DL-Valine	0·050	6	13·6 ± 1·2
DL-Alanine	0·050	6	14·1 ± 1·5
DL-Methionine	0·050	6	14·4 ± 2·7
Glycine	0·050	4	15·2 ± 3·2
L-Leucine	0·050	6	14·8 ± 1·5
DL-Threonine	0·050	6	15·1 ± 2·7
'Mean'	0·050 (Except cysteine)	9	14·0 ± 0·9
(2) Small significant effect on solubility of estradiol-17β			
L-Proline	0·050	6	15·6 ± 2·1
L-Histidine Monohydrochloride	0·050	6	15·5 ± 0·9
(3) Pronounced effect on solubility of estradiol-17β			
L-Arginine Monohydrochloride	0·050	6	16·4 ± 0·5
DL-Aspartic Acid	0·040	6	16·7 ± 1·2
DL-Tryptophan	0·010	6	18·4 ± 0·9
L-Glutamic Acid	0·040	6	19·1 ± 1·9
L-Lysine Monohydrochloride	0·050	6	19·1 ± 1·0
L-Tyrosine	0·005	6	24·2 ± 1·8
(4) Varying ionic strength of buffer or salt solutions. No amino acids added			
Phosphate buffer	0·050	10	13·8 ± 1·7
Phosphate buffer	0·100	14	13·2 ± 0·6
Phosphate buffer	0·201	8	12·3 ± 0·9
Sodium chloride	0·020	3	14·0
	0·050	3	13·9
	0·100	8	13·3 ± 1·4
	0·200	3	12·5
	0·400	3	10·4
	0·600	3	9·5

liter, pS_0 is the negative logarithm of the solubility in water in moles per liter and Γ is the ionality (double ionic strength) in moles per liter[6]. From the results in Table 1 a regression equation $Y = 0{\cdot}30x + 4{\cdot}85$ is obtained and a solubility at zero ionic strength of $14{\cdot}3 \times 10^{-6}$ moles/l.. Bischoff and Philhorn[6] give a K-value of approximately 0·20 with a solubility at zero ionic strength of $10{\cdot}5 \times 10^{-6}$ moles/l. The difference is probably due to methodology: Bischoff and Philhorn used a bioassay for the estimation of estradiol-17β while in the present investigation the Kober reaction was performed.

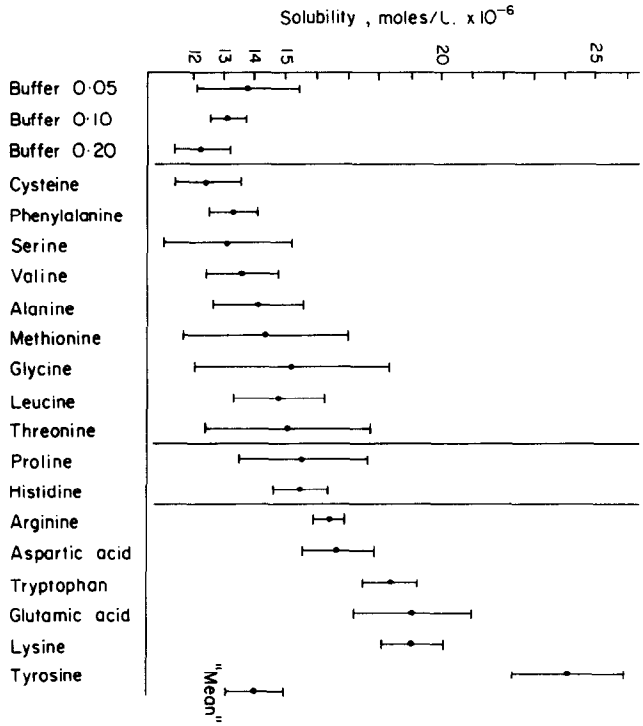

Fig. 2.

Although the standard deviation from the mean solubilities is quite large (average 10 per cent) two groups of amino acids clearly emerge, if the solubility of estradiol-17β in amino acid-buffer solutions is compared with that obtained in buffer alone: (1) those which have no influence on the solubility of estradiol-17β, (alanine, cysteine, glycine, leucine, methionine, phenylalanine, serine, threonine, valine); (2) some amino acids which increase the solubility of estradiol-17β considerably, namely arginine, aspartic acid, glutamic acid, lysine, tryptophan and tyrosine. Proline and histidine seem to increase the solubility of estradiol-17β to some extent. It is interesting to compare these results with those obtained for testosterone[1]. The solubility of both estradiol-17β and testosterone is increased by tyrosine, trytophan and the basic amino acids. Phenylalanine which increased the solubility of testosterone has no effect on estradiol-17β solutions. On the other hand glutamic acid and aspartic acid which caused a pronounced increase in the solubility of estradiol-17β have no influence on testosterone solutions. Proline in both cases appeared to slightly increase the solubility while no such influence could be observed for methionine in the case of estradiol-17β.

More studies involving a variety of steroids are necessary to establish the nature of steroid–amino acid interactions.

REFERENCES

1. D. Abelson, C. Depatie and V. Craddock: *Arch. Biochem.* **91** (1960) 71.
2. S. G. Korenman: *J. clin. Endocr.* **28** (1968) 127.
3. E. V. Jensen, H. J. Jacobson, J. W. Flesher, N. N. Saha, G. N. Gupta, S. Smith, V. Colucci, D. Shiplacoff, H. G. Neumann, E. R. DeSombre and P. W. Jungblut: in *Steroid Dynamics* (Edited by T. Nakad, G. Pincus and J. Tait). Academic Press, New York (1966) p. 131.

4. J. B. Brown: in *Advances in Clinical Chemistry* (Edited by H. Sobotka and C. P. Stewart). Academic Press, New York, Vol. 3 (1960) p. 157.
5. *Biochemists' Handbook* (Edited by C. Long, E. J. King and W. M. Sperry). E. and F. N. Spon, London (1961) p. 32.
6. F. Bischoff and H. R. Philhorn: *J. biol. Chem.* **174** (1948) 663.
7. F. Bischoff and R. E. Katherman: *Am. J. Physiol.* **152** (1948) 189.