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Interaction of Amino Acids with Bilirubin—I

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Abstract: Interaction of various amino acids (e.g. *l*-Histidine, *l*-Aspartic acid, *l*-Tyrosine, *l*-Tryptophan, *l*-Arginine) with bilirubin has been studied. It is hypothesized that the interaction is a chemical rather than enzymatic one, resulting in suppression of bilirubin, as has been found in *in vivo* studies carried out by Towne, Hamilton and Stemphenson.[1]

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Introduction

It has been claimed that the adminstration of amino acids suppressed the upper gastrointestinal secretions including bilirubin in dogs.[1] Of Interest is the suppression of bilirubin output after amino acid infusion. No precise mechanisn of action was suggested by these experiments. Our interest in bilirubin is part of investigations being carried out in these laboratories for the complete assignment of the electronic absorption spectrum of urine.[2] The present study points towards a direct interation of amino acids with bilirubin *in vitro*.

Materials and Methods

Amino acids used were obtained from BDH and bilirubin from Merck. All other chemicals used were of BDH, Analar grade. The spectra were recorded on a Beckman UV-Visible spectrophotometer. These experiments were carried out in chloroform and water (pH $>$ 8) to represent non-aqueous (e.g. lipids) and aqeous environments in the biological systems. In case of water, pH $>$ 8 was obtained by addition of sodium hydroxide and hydrochloric acid so as to have both Na^+ and Cl^- ions in the medium for their obvious importance in *in vivo* studies.

Preparation of Solutions

Saturated solutions of *l*-Histidine, *l*-Aspartic acid, *l*-Arginine, *l*-Tyrosine and *l*-Tryptophan were prepared by dissolving the amino acid in excess in cold water and filtering out the excess.

Bilirubin is insoluble in water at neutral pH but is soluble at pH $>$ 8. Sodium hydroxide and hydrochloric acid were used to obtain an aqueous solution of bilirubin at pH $\sim$ 8.5.

Solution for Recording Spectra

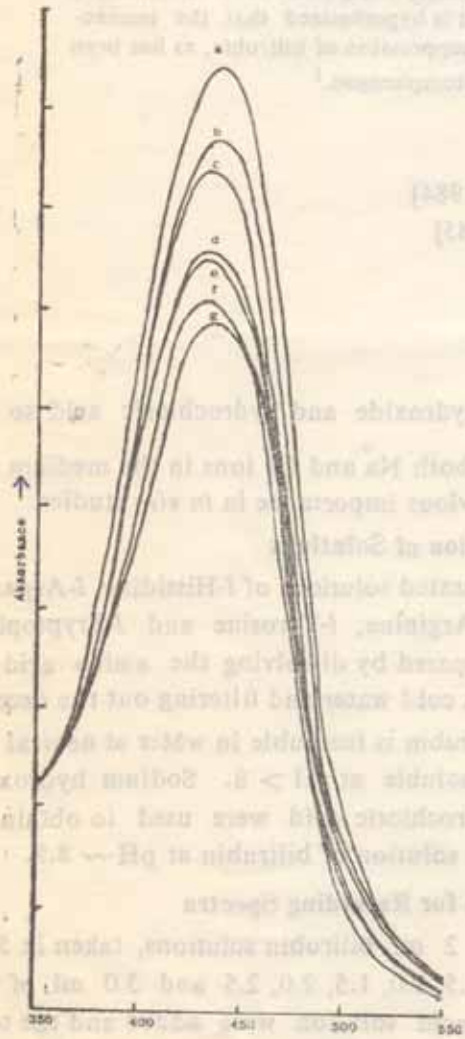
To 2 ml bilirubin solutions, taken in 5 ml flasks, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml of the amino acid solution were added and the total volume was made up to mark in each case by adding respective solvent. 3 ml of this reaction mixture was taken into sectrophotometric cell of 1 cm path length to record spectra in visible region. Equal volumes of the saturated solutions

of each amino acid were mixed together to look at the combined effect. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml of this mixed solution were added to bilirubin solution (2 ml) and spectra were recorded after making up the volume to 5 ml by adding the respective solvent.

The molar absorptivity was plotted against the volume of the amino acid solution added.

Results and Discussion

Bilirubin solution absorbs at 435 nm and molar absorptivity is found to be 46773.5. Figures 1,2 and 3, show absorbance versus



Wavelength in nm. ⟶

Fig. 1

Fig. 1. Electronic absorption spectrum of bilirubin after addition of *l*-Aspartic acid.

a = Pure bilirubin.

b = Bilirubin + 0.5 ml of saturated solution of *l*-Aspartic acid.

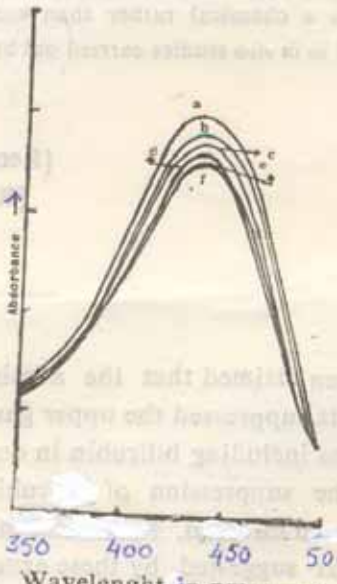
c = Bilirubin + 1.0 ml of saturated solution of *l*-Aspartic acid.

d = Bilirubin + 2.0 ml of saturated solution of *l*-Aspartic acid.

e = Bilirubin + 1.5 ml of saturated solution of *l*-Aspartic acid.

f = Bilirubin + 2.5 ml of saturated solution of *l*-Aspartic acid.

g = Bilirubin + 3.0 ml of saturated solution of *l*-Aspartic acid.



Wavelenght in nm. ⟶

Fig. 2

Fig. 2. Electronic absorption spectrum of bilirubin after addition of *l*-Arginine.

a = Bilirubin + 2.5 ml of saturated solution of *l*-Arginine.

b = Bilirubin + 2.0 ml of saturated solution of *l*-Arginine.

c = Bilirubin + 1.0 ml of saturated solution of *l*-Arginine.

d = Bilirubin + 1.5 ml of saturated solution of *l*-Arginine.

e = Bilirubin + 0.5 ml of saturated solution of *l*-Arginine.

f = Pure Bilirubin.

Wavelength in nm. ⟶

Fig. 3

Fig. 3. Electronic absorption sepectrum of bilirubin after addition of mixture of amino acids solution.

a = Pure Bilirubin.

b = Bilirubin + 0.5 ml of mixture of amino acids Solution.

c = Bilirubin + 1.0 ml of mixture of amino acids Solution.

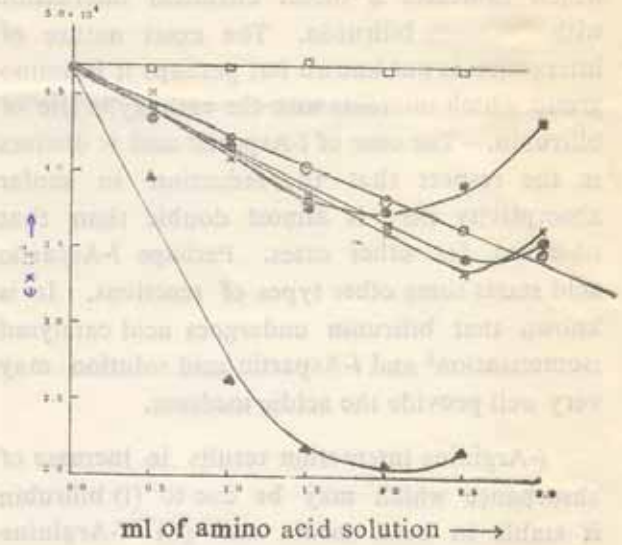
d = Bilirubin + 1.5 ml of mixture of amino acids Solution.

e = Bilirubin + 2.0 ml of mixture of amino acids Doluyion.

f = Bilirubin + 2.5 ml of mixture of amino acids Solution.

g = Bilirubin + 3.0 ml of mixture of amian acids Solution.

wavelength plots after mixing *l*-Aspartic acid, *l*-Arginine and mixture of amino acids as described earlier, respectively. Figure 4 shows plots of molar absorptivity after addition of amino acids to bilirubin solutions. The percentage change in molar absorptivity with addition of different amino acids is given in Table 1. Similar results were obtained with both the solvents, i.e., chloroform and water.



ml of amino acid solution ⟶

Fig. 4. Plots of Molar Absorptivity (after mixting amino acids with bilirubin) versus concentration of amino acids.

× ℓ-Histidine

△ ℓ-Aspartic Acid

Fig. 4 ● ℓ-Tyrosine

■ ℓ-Tryptophan

⊙ Mixture of amino acid

□ ℓ-Arginine

Table 1

S. No.	Amino Acid added.	% age change in Molar Absorptivity
1.	*l*-Aspartic acid.	−55.42
2.	*l*-Histidine	−28.57
3.	*l*-Tyrosine	−26.77
4.	*l*-Tryptophan.	−23.93
5.	*l*-Arginine.	+ 9.64
6.	Mixture of 1-5	− 25.64

It is obvious from Table 1 that except for the addition of *l*-Arginine, every amino acid studied results in decrease of molar absorptivity,

which indicates a direct chemical interaction with bilirubin. The exact nature of interaction is not known but perhaps it is amino group which interacts with the carboxylic site of bilirubin. The case of *l*-Aspartic acid is distinct in the respect that the reduction in molar absorptivity here is almost double than that observed for other cases. Perhaps *l*-Aspartic acid starts some other types of reactions. It is known that bilirubin undergoes acid catalysed isomerisation[3] and *l*-Aspartic acid solution may very well provide the acidic medium.

l-Arginine interaction results in increase of absorbance which may be due to (i) bilirubin is stable in basic media and (ii) *l*-Arginine provides an additional guanidino group ; i.e. $-NH-(NH_2)\,C=NH$.

It is, therefore, suggested that while estimating bilirubin in body fluids spectrophotometrically, the presence of different metabolites may be taken care off.

The conclusion of Towne and coworkers[1] that suppression of bilirubin on infusion of amino acids is a result of enzymatic action was an indirect one, not based on experimental evidences. The present studies lead to the conclusion that the observed suppression of bilirubin may be due to the direct interaction of amino acids with bilirubin. Similar observations have been made by Rutkowski,[4] who has postulated a direct interaction of albumin with bilirubin. Nevertheless, the possibility of some enzymatic interaction can not be completely ruled out.

Acknowledgement

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