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The Effect of Iodination on the Binding of Thyroxine by Human Serum Albumin

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The binding of thyroxine to human serum albumin and to albumins iodinated to different extents has been studied and the influence of a change in *p*H examined. It appears that the tyrosyl residues in albumin are involved in the sites of binding and that the dissociation of the phenolic groups inhibits uptake of thyroxine. In the presence of a dissociated phenolic group, thyroxine binds less readily to a partially iodinated than to an uniodinated or a completely iodinated protein. This has been tentatively explained as a steric hindrance to binding on a charged monoiodotyrosyl residue. In the absence of a negative charge, a completely iodinated protein binds thyroxine with the greatest affinity suggesting that the uncharged diiodotyrosyl residue is best adapted to thyroxine binding.

The fact that thyroxine is bound very strongly to a specific protein in serum is well established.[1] It is also known that serum albumin binds thyroxine, although less strongly than the specific protein.[2] Very little quantitative work has been done by way of elucidating the nature of these complexes. Lein[3] has used the method of the spectrum shift of thyroxine bound to bovine serum albumin, and while the details of his experiment have not been published, he appears to have obtained significantly quantitative results. Larson and Albright[4] have used the method of displacement of bound thyroxine from the thyroxine–specific protein complex by thyroxine analogs and have discussed the relative importance of functional groups and spatial arrangements on the binding capacities.

In this paper we have investigated the quantitative effects of iodination of human serum albumin on its capacity to bind thyroxine.

Experimental

Materials and Methods.—The human serum albumin (HSA) used was a 25% solution of normal albumin, salt-free, obtained from the Cutter Laboratories. The solution was 0.02 *M* in sodium acetyl tryptophanate and sodium caprylate, which act as stabilizers. In all the iodinated albumins extensive dialysis was involved before binding of thyroxine was studied so that these materials were eliminated. For one set of data on uniodinated albumin, 25 ml. of protein solution was dialyzed against 1 liter of distilled water for 24 hr. with 3 changes of water in order to remove these stabilizers.

The thyroxine used was sodium L-thyroxinate obtained from Nutritional Biochemical Corporation, and the I-131-labeled thyroxine was a chromatographically pure sample obtained from Abbott Laboratories.

The method suggested by Klotz,[5] in which the shift in an absorption peak of thyroxine is observed after absorption by the protein, was employed. A 1-cm. length quartz cuvette was used in conjunction with a Beckman spectrophotometer, model DU. Thyroxine shows a convenient peak in its ultraviolet spectrum at 331 mμ, where the absorption by the protein is minimal. Systems of protein–thyroxine–buffer were made up so that various concentrations of protein were found in a fixed concentration of thyroxine. The buffer was a 0.075 *M* phosphate system at *p*H 11.2. The optical densities (OD) of these systems were measured at 331 mμ against a blank of exact duplicates without thyroxine. The OD of unbound thyroxine was taken as that found in the system containing no protein, and the OD of bound thyroxine as the highest value reached in the presence of very high protein concentrations.

Since iodinated proteins have an increasingly high absorption at 331 mμ, as the iodine content of the protein increases, the above-mentioned method becomes useless for analysis of highly iodinated proteins. The method of equilibrium dialysis was therefore used for these latter systems; no correction for the Donnan membrane equilibrium was considered necessary, since maximum protein concentrations were of the order of 1% and in a buffered system of high ionic strength. Dialysis sacks and dialysate were shaken at room temperature (25°) for periods ranging from 24 hr. to 3 days, depending upon the protein concentration. The equilibrium concentration of thyroxine was determined either by measuring the OD of the dialysate at 331 mμ or by using labeled thyroxine and counting aliquot samples of the dialysate. A correction was applied for the uptake of thyroxine by the cellophane sacking, this being obtained by direct measurement on a duplicate sack containing no protein.

Iodination of the serum albumin was carried out according to Hughes and Straessle,[6] using a carbonate–bicarbonate buffer at *p*H 10.0 (unless otherwise stated) with subsequent dialysis at 5° to remove excess electrolyte. According to Hughes and Straessle,[6] iodination between *p*H 9.0 and 10.15 gives rise to 90–95% of the consumed iodine as organically bound iodine. Moreover, of the organic iodine, about 25 atoms per albumin molecule will be bound in the 3,5-positions of the tyrosyl residues before other groups, most probably histidyl, become involved in iodination. Tryptophan only becomes involved in oxidation by iodine when large excesses of iodine are present, and we have been careful to avoid this condition. For the purposes of this investigation, we have assumed 100% organically bound iodine and calculated the number of iodine atoms per mole of albumin on the basis of how much iodine was consumed during the iodination.

Protein concentrations were determined either by a quantitative biuret reaction or by drying aliquots of the solutions and direct weighing of the residues. Counting of radioactive samples was performed in a Nancy Wood well-type scintillation counter and scaler. The systems examined at *p*H's 9.4 and 7.05 were allowed to approach dialysis equilibrium from both sides of the equilibrium by using duplicate systems; these were shaken gently at room temperature for 4 hr. and the mean of the thyroxine concentrations found in the dialysates taken as the equilibrium value. The value in duplicate systems did not in general differ by more than 5%.

Results

Figure 1 shows the results on 4 different systems examined at *p*H 11.2. Results are in general not precise enough to allow for a detailed analysis of binding characteristics, such as maximum binding capacity and association constants of the protein–thyroxine complex, but they are not inconsistent with the figures of 4 and 8 moles thyroxine per mole albumin given by Lein[3] for the 2 types of binding sites on bovine serum albumin.

Table I summarizes the data obtained at *p*H 9.4 and 7.05, phosphate buffer. In these experiments,

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(2) J. Robbins, J. E. Rall and M. L. Petermann, *J. Clin. Invest.*, **33**, 959 (1954).

(3) A. Lein, *Federation Proc.*, **11**, 91 (1952).

(4) F. C. Larson and E. C. Albright, *Endocrinology*, **56**, 737 (1955).

(5) I. M. Klotz, THIS JOURNAL, **68**, 2299 (1946).

(6) W. L. Hughes, Jr., and R. Straessle, *ibid.*, **72**, 452 (1950).

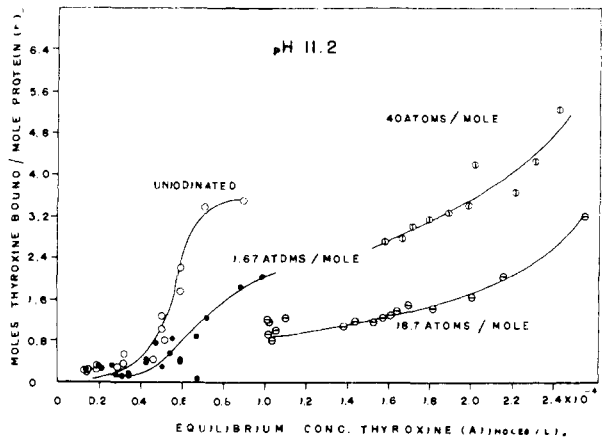

Fig. 1.—The binding of thyroxine by albumins of different iodine content, at *p*H 11.2.

exactly similar quantities of protein were iodinated at *p*H 9.4 to different degrees by addition of exact quantities of iodinating solution[6] and left for 24 hr. at 6° when no iodine could be detected by a starch indicator. These solutions of iodo-protein were then used directly in dialysis equilibrium systems as already described. Exactly similar concentrations of thyroxine were used for each degree of iodination.

TABLE I

EFFECT OF INCREASING IODINATION ON THYROXINE-BINDING

Atoms I_2 per mole albumin	*p*H	Moles Tx bound per mole albumin (r)	Equilibrium concn. Tx (moles/l. × 10^7) (A)	r/A (× 10^{-5})
0	9.4	4.87	435	1.12
2	9.4	4.91	430	1.14
4	9.4	4.35	490	0.89
6	9.4	4.43	485	.91
10	9.4	4.09	525	.78
20	9.4	4.13	520	.79
30	9.4	4.40	490	.90
40	9.4	4.35	495	.88
0	7.05	0.145	4.83	3.00
2	7.05	.145	4.83	3.00
4	7.05	.169	4.53	3.72
6	7.05	.169	4.56	3.70
10	7.05	.165	4.59	3.59
20	7.05	.172	4.51	3.81
30	7.05	.190	4.21	4.50
40	7.05	.210	4.06	5.16

Discussion

It would appear from Fig. 1 that the introduction of iodine atoms on the tyrosyl residues of human serum albumin tends first to inhibit the uptake of thyroxine at a *p*H of 11.2. For the lowest degree of iodination it is certain that some oxidation of sulfhydryl groups is involved as well as iodination,[6] but by the time 18.7 atoms of iodine are bound to the albumin molecule, a considerable part of the 18 tyrosyl residues[7] will be converted to monoiodotyrosyl with the formation of some diiodotyrosyl. On the other hand, with the introduction of 40 atoms of iodine per mole of albumin, it is probable that most of these tyrosyl residues will be converted to diiodotyrosyl and we see that the capacity to bind thyroxine is reintroduced to the albumin molecule.

Inasmuch as the phenolic group of tyrosyl residues in polypeptides has a pK value (50% dissociation) of about 11 and of diiodotyrosyl residues of about 8.5,[8,9] this change in affinity for thyroxine would seem to reflect a steric hindrance to binding on charged monoiodotyrosyl residues at *p*H 11.2, where essentially all phenolic groups are dissociated.

Katchalski and Sela[8] have titrated polytyrosine and polydiiodotyrosine as well as copolymers of these with other amino acids. Their results show that, like HSA, all phenolic groups are available for titration. Quantitatively, they show that, depending upon the extent of iodination, at *p*H 11.2 from 80 to 100% of the phenolic groups are dissociated, at *p*H 9.4 from 25 to 80% and at *p*H 7.05 from 0 to 10%.

The data in Table I can be readily interpreted in the light of these values. The very much greater values of r/A at *p*H 7.05 compared to *p*H 11.2 show the effect of a negative charge at the tyrosyl residues of uniodinated, partially iodinated and completely iodinated protein in inhibiting binding of thyroxine. This is not surprising since it is the thyroxinate ion that must be bound at *p*H 11.2. Moreover, the higher value of r/A for the completely iodinated, as compared to the partially and uniodinated protein, would indicate the higher affinity of undissociated diiodotyrosyl residues for thyroxine as compared to undissociated monoiodotyrosyl and tyrosyl residues.

At *p*H 9.4, we have incomplete dissociation for all states of iodination, and this is reflected in the intermediate values of r/A when compared with higher and lower *p*H's. The relative constancy of these r/A values may be attributed to the opposing effects of increasing iodination and increasing dissociation.

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