The specific binding of the thyroid hormones to matrix isolated from rat liver nuclei

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Summary

Specific binding sites for the thyroid hormones have been demonstrated in the liver nuclear matrix, a structural framework of the nucleus. When labelled 3,5,3′-tri-iodo-L-thyronine ([^{125}I]T_3) is injected into rats, 5% of the total nucleus bound T_3 is bound to the matrix after 1 hour. However, when either isolated nuclei or isolated nuclear matrices were incubated with [^{125}I]T_3 *in vitro*, a 3- to 7-fold greater number of specific T_3 binding sites were revealed in the nuclear matrix. The properties of the matrix-associated thyroid hormone binding sites were investigated *in vitro*. These binding sites showed limited capacity and high affinity for T_3; the equilibrium association constant (K_a) was $1,3 \times 10^9$ M^{-1} and the binding capacity was 20,2 fmol T_3 per 100 μg matrix protein.

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An elaborate structural network has been isolated from the nuclei of a variety of eukaryotic cells.[1-5] This nuclear framework is termed the nuclear matrix and is composed of over 95% protein and very small quantities of DNA, RNA and phospholipid.[2] The protein fraction consists essentially of three major non-histone peptides of molecular weight range 60 000 - 70 000 daltons, as demonstrated by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis.[2]

The nuclear matrix appears to be associated with certain functional aspects of the nucleus. In a recent study on regenerating liver, Pardoll *et al.*[6] presented evidence that the nuclear matrix provides fixed sites for the attachment of DNA replication complexes and that the DNA is reeled through these sites as it is replicated. Furthermore, it has been demonstrated by Allen *et al.*[7] that the phosphorylation of matrix proteins is markedly increased during liver regeneration and that the enhanced phosphorylation precedes the onset of DNA synthesis. These findings suggest that the phosphorylation of specific matrix proteins may be important in the regulation of nuclear DNA synthesis. Barrack *et al.*[3,8] have demonstrated the presence of salt-resistant oestrogen binding sites in rat uterine nuclei which appear to be associated with the nuclear matrix. These salt-insoluble binding sites for oestrogen are of particular interest in view of the suggested correlation between oestrogen binding to these sites and oestrogen-induced uterine growth in the rat.[9] The report by Honma *et al.*[10] also suggests that salt-resistant binding sites for the steroid hormones may play an important role in nuclear function. They demonstrated that dexamethasone-sensitive murine leukaemic myeloblasts contain salt-resistant nuclear binding sites for glucocorticoids, whereas the nuclei of certain dexamethasone-resistant cells contain only salt-extractable hormone binding sites. These findings suggest that the hormone binding sites present on nuclear matrices may be involved in the responsiveness of normal and neoplastic cells to specific steroid hormones.

Putative receptors for 3,5,3′-tri-iodo-L-thyronine (T_3) have been demonstrated in the cell nuclei of thyroid hormone-responsive tissues and these receptors appear to be closely linked to the expression of the thyroid hormone effect.[11-17] About 60% of the T_3 receptors can be extracted from these nuclei with 0,4M KCl.[15,18] However, even salt concentrations greater than 1M are only able to extract about 80% of the total T_3 receptors present in either GH_1 cell nuclei or rat liver nuclei.[15] In view of the increasing evidence suggesting a functional role of the nuclear matrix binding sites for the steroid hormones, we decided to investigate the nature of the salt-resistant T_3 binding sites. Such an investigation may provide new insight into the mechanism of action of the thyroid hormones.

Materials and methods

Materials

^{125}I-labelled T_3, L-thyroxine (T_4) and 3,3′,5′-tri-iodo-L-thyronine (rT_3) with high specific activity (450-1 250 mCi/mg) were obtained from Abbott Radiopharmaceuticals or New England Nuclear. According to the manufacturers' specifications, all of these isotopes were at least 96% pure and they were used within 7 - 10 days after arrival. Enzymes used were electrophoretically purified DNase I from Sigma and 5 times crystallized pancreatic RNase from Miles Laboratories. Wistar rats were supplied by the National Research Institute for Nutritional Diseases of the South African Medical Research Council.

Methods

Isolation of liver nuclei. Liver nuclei, purified through 2,2M sucrose, were prepared from male Wistar rats (150 - 180 g) according to the procedure of Berezney and Coffey,[2] except that 5 mM $NaHSO_3$ was added to all buffers. The purified nuclei from a single liver were washed twice with 5 ml STM buffer (0,25M sucrose, 5 mM $MgCl_2$, 10 mM tris, pH 7,4 at 22°C) and then washed once with 5 ml STM buffer containing 1% Triton X-100. The washed nuclei were rinsed with 5 ml STM buffer and then checked for contaminating cytoplasmic debris by electron microscopy and found to be devoid of any contamination.

Isolation of nuclear matrix. Nuclear matrix was prepared from the washed nuclei, essentially according to the method described by Barrack *et al.*[3] All centrifugations were at 800 *g* for 10 - 20 minutes. The nuclei from a single liver were suspended in

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5 ml STM buffer containing DNase I 50 μg/ml and RNase 100 μg/ml. The nuclear suspension was incubated for 30 minutes at 18°C with gentle stirring and then centrifuged. The pellet was rinsed by gently suspending it in 5 ml SLM buffer (0,25M sucrose, 0,2 mM $MgCl_2$, 10 mM tris, pH 7,4 at 22°C), centrifuged and extracted twice for 15 minutes with SLM buffer containing 2M NaCl (4 ml used for first extraction and 2 ml for second extraction). The pellet was rinsed with 2 ml STE buffer (0,25M sucrose, 1,5 mM EDTA, 10 mM tris, pH 7,4 at 22°C), centrifuged and resuspended in 0,5 ml STE buffer. This final suspension is the nuclear matrix.

Labelling of nuclear matrix with (^{125}I)T_3. Matrix labelled with [^{125}I]T_3 *in vivo* was prepared as described from the livers of rats which had received a single subcutaneous injection of 95μCi [^{125}I]T_3 and been killed after 1 hour. [^{125}I]T_3-labelled matrix was also obtained by incubating the purified nuclei with 5 nM [^{125}I]T_3 for 16 hours at 4°C in 5 ml STM buffer. After incubation, the nuclei were washed twice with 1% Triton X-100 in STM buffer and the labelled nuclear matrix was isolated according to the procedure described. The specific [^{125}I]T_3 binding to matrix *in vitro* was determined by subtracting the matrix binding obtained in a parallel experiment for nuclei incubated in the presence of a 200-fold molar excess of non-radioactive T_3. The direct binding of T_3 to nuclear matrix was measured by incubating isolated matrix (about 600 μg matrix protein) in STE buffer, pH 8,0 at 22°C, containing 5 mM 2-mercapto-ethanol for 3 hours at 20°C with 1 - 5 nM [^{125}I]T_3. Specific [^{125}]T_3 binding to the nuclear matrix was determined by subtracting the [^{125}I]T_3 binding obtained in the presence of a 200-fold molar excess of T_3.

Analytical procedures. SDS polyacrylamide electrophoresis of proteins was performed on 7,5 - 15% acrylamide slab gradient gels using the buffer system described by Laemmli.[19] For electron microscopy, matrices were fixed in cold 5% gluteraldehyde containing 0,1M sodium phosphate buffer, pH 7,2. Post-fixation was with 1% osmium tetroxide buffered at pH 7,4; after dehydration samples were embedded in Epon-Araldite, cut and mounted on 400-mesh copper grids and stained with uranyl acetate and lead citrate. Electron micrographs were taken with a Philips EM 300 electron microscope at 40 kV.

Radioactivity (^{125}I) was determined with a Packard Auto Gamma spectrometer with a counting efficiency of 72%. Protein concentrations were measured by the microbiuret method[20] and DNA was determined by the modified method of Burton.[21]

Results

Characterization of the nuclear matrix

Sequential treatment of purified rat liver nuclei with Triton X-100, combined DNase I and RNase digestion followed by extraction with 2M NaCl, yielded a residual nuclear superstructure termed the nuclear matrix (Fig. 1). The electron micrograph reveals that the liver nuclear matrix which was isolated by this procedure is composed of residual elements of the nuclear membrane and nucleolus and a network of interconnecting fibres. This is in agreement with the observations of Berezney and Coffey.[2] Similarly, analyses of the protein composition of the matrix by SDS polyacrylamide gel electrophoresis showed that, although the matrix consists of many proteins, it is primarily composed of three major polypeptides ranging in molecular weight from 60 000 to 70 000 daltons (Fig. 2). Quantitative DNA and protein analyses performed on the matrix indicated that it consists of less than 3% of the total nuclear DNA and about 15% of the total nuclear protein. A second treatment of the salt-extracted matrix with DNase I removed very little of the matrix-associated DNA. This is in agreement with the findings of Shaper *et al.*[22] who reported that a small amount of DNA remains closely associated with the protein matrix.

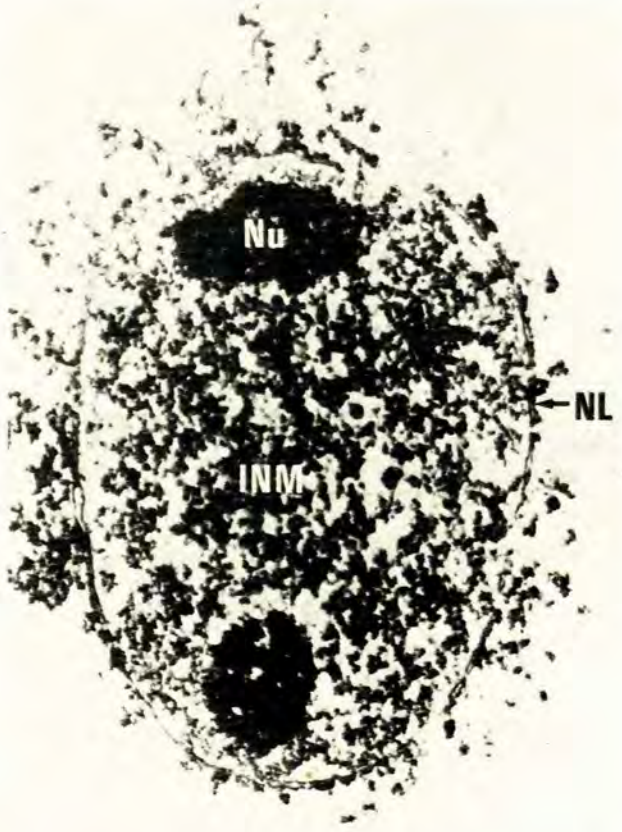

Fig. 1. Ultrastructure of the nuclear matrix isolated from rat liver. The matrix consists of an outer nuclear lamella (NL); residual nucleoli (Nu) and a network of interconnecting fibres (INM) (magnification × 15 000).

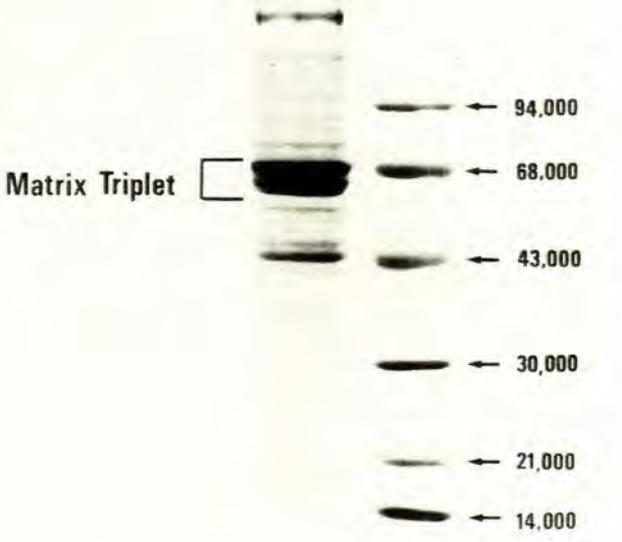

Fig. 2. Electrophoretic profile of stained polypeptide components of isolated nuclear matrix from rat liver. The migration of proteins of known molecular weights is shown on the right. The bulk of the matrix proteins occurs as a triplet at about 68 000 daltons.

Identification of T_3 binding sites in matrix

One hour after the injection of [^{125}I]T_3 into a male rat, 884 fmol T_3 were bound specifically to the nuclei isolated from a single liver (Table I, A). After fractionating these nuclei according to the procedure described under 'Materials and methods', 5% (49 fmol) of total nuclear T_3 binding remained associated with the salt-insoluble nuclear matrix. However, when the nuclear matrix was further subjected to an *in vitro* incubation with 5 nM [^{125}I]T_3, an additional 315 fmol T_3 bound specifically to the matrix. This additional T_3 binding was resistant to high salt concentrations, about 70% remaining bound after a further extraction with 2M NaCl.

When isolated liver nuclei were incubated with 5 nM [^{125}I]T_3 under the conditions described in 'Materials and methods', 1 240 fmol T_3 bound specifically to nuclei derived from a single liver (Table I, B). If these nuclei were subjected to the identical fractionation procedures as the *in vivo* labelled nuclei, 17% (206 fmol) of the total nuclear T_3 binding remained in the salt-insoluble matrix. In relation to total nuclear T_3 binding, nuclear matrix labelled by incubating isolated nuclei with [^{125}I]T_3 revealed a 3 times greater number of specific T_3 binding sites than matrix labelled *in vivo*. In contrast, labelling of the total soluble nuclear extract is equally efficient by both the *in vivo* and *in vitro* procedures (Table I). The apparent mean T_3 binding capacity of the matrix obtained from incubation of isolated matrix with 5 nM T_3 *in vitro* was 355 fmol (Table I, A and B). Therefore, labelling of the intact liver nuclei *in vitro* under the conditions described can saturate approximately 60% of the total T_3 binding sites present in the nuclear matrix.

T_3 binding characteristics *in vitro*

When increasing amounts of non-radioactive T_3 were incubated together with 1 nM [^{125}I]T_3, a progressive decrease in the percentage of [^{125}I]T_3 bound to the matrix was observed. In the representative experiment shown in Fig. 3, the percentage [^{125}I]T_3 decreased to about 30% of the tracer binding when the matrix was incubated with 200 nM T_3. Higher concentrations of T_3 (up to 1 000 nM) did not result in any further decrease of [^{125}I]T_3 bound, and the 30% value was considered to represent nonspecific T_3 binding. In our experiments any binding obtained in the presence of a 200-fold excess of non-radioactive T_3 was regarded as nonspecific and was subtracted from the total binding.

The kinetics and stability of T_3 binding to nuclear matrix in the *in vitro* systems were studied. Fig. 4 shows the time course of T_3 binding to matrix at 4°C and 37°C. The T_3 binding was optimal at 20°C with an equilibrium being reached at 3 - 6 hours. Equilibrium binding was maintained for at least 12 hours, suggesting that T_3 binding to the matrix was very stable at 20°C. Optimal binding was also achieved at 4°C, but at this temperature equilibrium was only obtained after 12 - 18 hours. At 37°C the T_3 complexes formed within short times were progressively destroyed so that optimum binding was never achieved. Furthermore, the nonspecific binding of T_3 to the

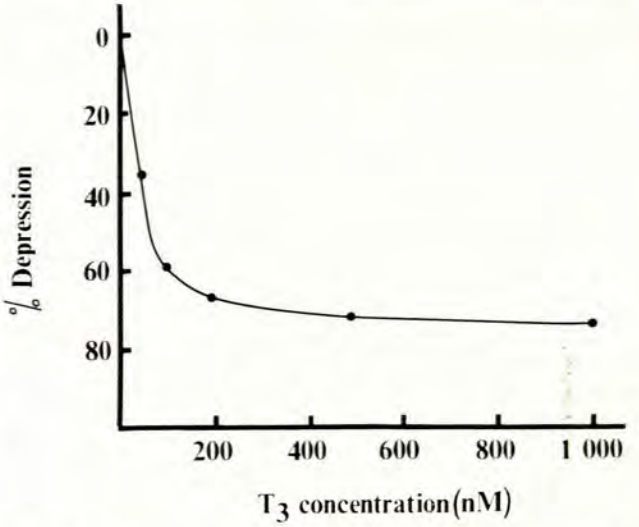

Fig. 3. Displacement of tracer [^{125}I]T_3 bound to nuclear matrix by increasing amounts of non-radioactive T_3. Matrix (93 μg protein) was incubated as described under 'Materials and methods' with 1 nM [^{125}I]T_3 and increasing amounts of non-radioactive T_3 up to 1 000 nM.

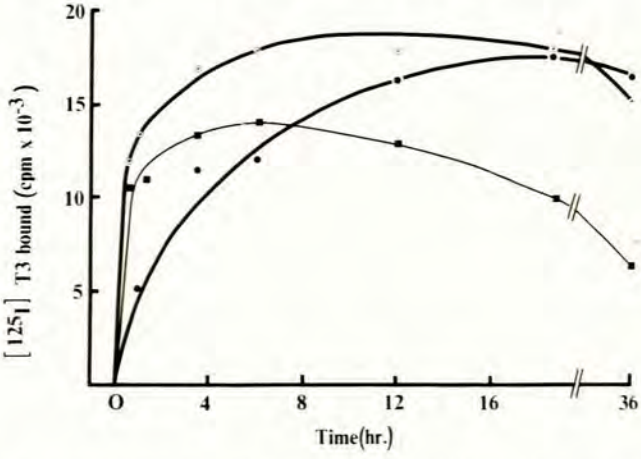

Fig. 4. Kinetics of binding of T_3 to nuclear matrix. Matrix (70 μg protein) was incubated for various intervals at 4° C (●—●), 20° C (Θ—Θ) and 37° C (■—■) with 5 nM [^{125}I]T_3 as described under 'Materials and methods'. The binding obtained with 1μM T_3 was subtracted for each assay.

TABLE I. DISTRIBUTION OF SPECIFIC T_3 BINDING SITES IN RAT LIVER NUCLEI LABELLED UNDER VARIOUS CONDITIONS

Experiment	Labelling procedure	Fraction	Specific T_3 binding (fmol/liver)
A1	[^{125}I]T_3 *in vivo*	Total nuclei	884
		Total soluble nuclear extract	870
		Insoluble nuclear matrix	49
A2	[^{125}I]T_3 *in vivo* then incubated with [^{125}I]T_3 *in vitro*	Insoluble nuclear matrix	364
B1	[^{125}I]T_3 labelling of nuclei *in vitro*	Total nuclei	1 240
		Total soluble nuclear extract	1 021
		Insoluble nuclear matrix	206
B2	[^{125}I]T_3 *in vitro*	Insoluble nuclear matrix	346

matrix was much greater at 37°C and represented 60% of the total binding after 3 hours. In contrast, the nonspecific binding at 4°C and 20°C was 30% or less at 3 hours.

The amount of T_3 specifically bound to the isolated matrix *in vitro* was proportional to the amount of added matrix protein up to at least 250 μg (Fig. 5). Also, T_3 binding to the matrix appeared to be independent of ionic strength and was not affected by NaCl concentrations up to 400 mM. An analysis of the effect of pH on the T_3 binding activity is shown in Fig. 6. It is observed that T_3 binding to the matrix is sensitive to pH, with optimum binding occurring at pH 8,0. The possible influence of 2-mercapto-ethanol on T_3 binding to the matrix was tested at pH 8,0 but was found to have no effect. This indicated that binding is independent of the redox state of the -SH groups of the matrix binding sites. To investigate the affinity and capacity of the nuclear matrix for T_3, increasing quantities of [^{125}I]T_3 (0,1 - 5 nM) were added to the incubation medium. The T_3 specifically bound to the matrix and the amount of unbound T_3 remaining at the end of the incubation were measured and the data were plotted according to the method of Scatchard.[23] The Scatchard plot (Fig. 7) showed that the matrix probably has a single class of specific T_3 binding sites with high affinity (equilibrium association constant (K_a) = 1,3 x $10^9 M^{-1}$). The binding capacity was also calculated from the plot and found to be 20,2 fmol T_3 per 100 μg matrix protein.

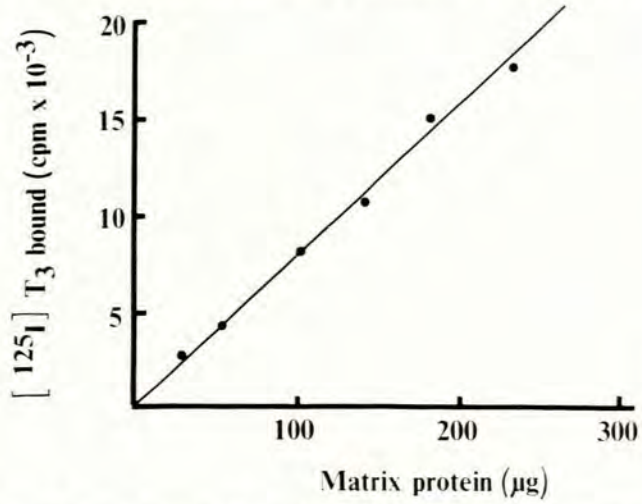

Fig. 5. Specific binding of T_3 as a function of the concentration of nuclear matrix added. Increasing amounts of nuclear matrix were incubated with 5 nM [^{125}I]T_3 under the conditions described under 'Materials and methods'. The binding obtained with 1 μM T_3 was subtracted for each assay.

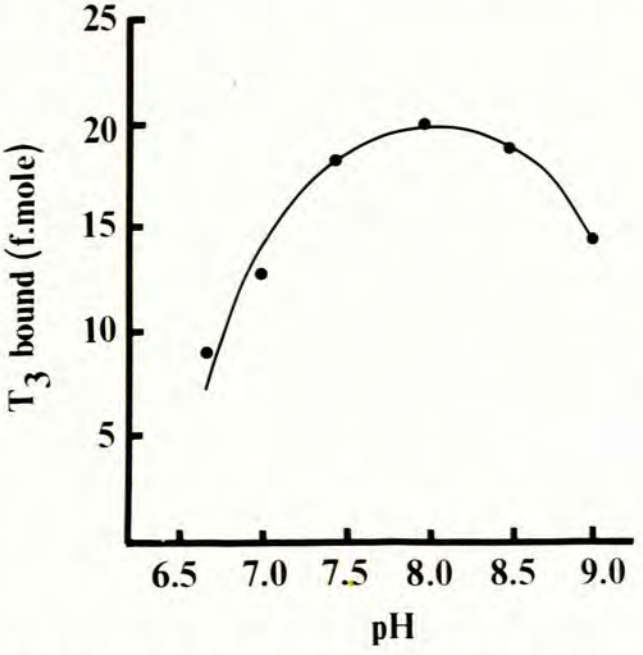

Fig. 6. Effect of pH on T_3 binding to nuclear matrix: 5 nM [^{125}I]T_3 was incubated with 103 μg matrix protein under the conditions described under 'Materials and methods', except that the pH of the buffer was varied. The binding obtained with 1 μM T_3 was subtracted for each assay.

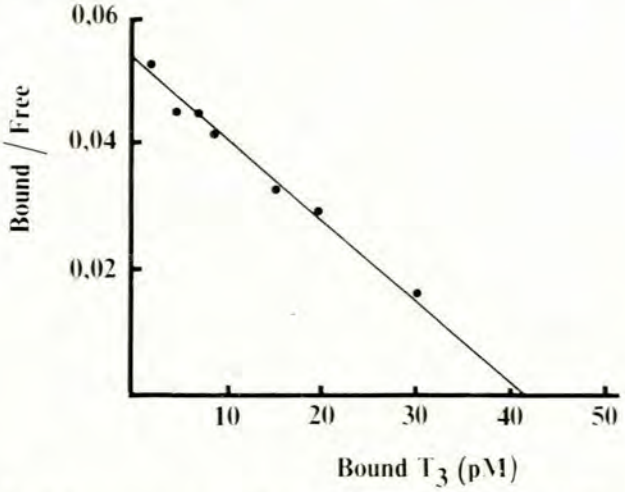

Fig. 7. Scatchard analysis of T_3 binding to nuclear matrix. Matrix (101 μg protein) was incubated with various concentrations of [^{125}I]T_3 ranging from 0,1 to 5 nM as described under 'Materials and methods'. The binding obtained with a 200-fold molar excess of T_3 was subtracted for each assay.

Iodothyronine binding characteristics *in vitro*

The nuclear matrix was shown to bind T_4, but it had a substantially lower avidity for T_4 than T_3 (Fig. 8). The T_4 binding was about 40% that of T_3. Furthermore, the matrix bound rT_3 to a small extent, the rT_3 binding being only 10% that of T_3. The binding of T_4 and rT_3 was optimal at 20°C and at that temperature the kinetics of binding was identical to T_3 so that equilibrium binding with T_4 and rT_3 was also reached at 3 - 6 hours (Fig. 8). The effect of pH on T_4 binding to nuclear matrix was determined and the pH curve was found to be entirely different from that obtained with T_3. The T_4 binding was

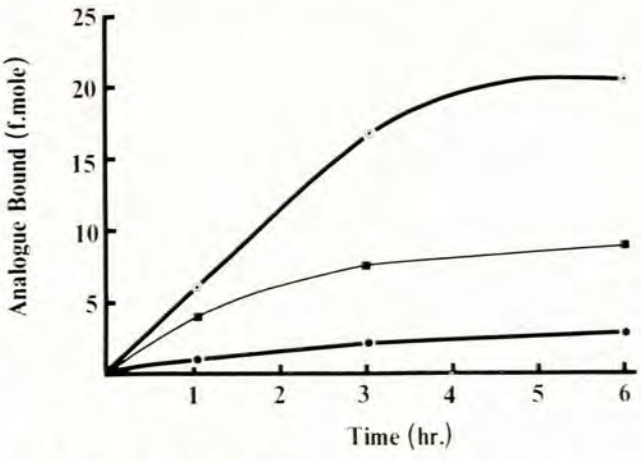

Fig. 8. Kinetics of specific binding of iodothyronine analogues to nuclear matrix: 5 nM [^{125}I]T_3 (Θ—Θ), 5 nM [^{125}I]T_4 (■—■) or 5 nM [^{125}I]rT_3 (●—●) were incubated with 103 μg matrix protein as described under 'Materials and methods'. The binding obtained with 1 μM T_3, T_4 or rT_3 was subtracted for each appropriate assay.

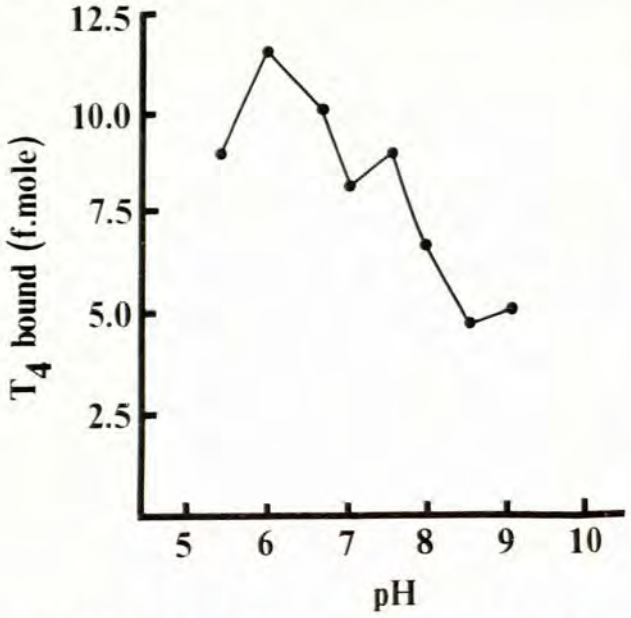

Fig. 9. Effect of pH on T_4 binding to nuclear matrix: 5 nM [^{125}I]T_4 was incubated with 103 μg matrix protein under the conditions described under 'Materials and methods', except that the pH of the buffer was varied. The binding obtained with 1 μM T_4 was subtracted for each assay. To obtain the pHs ranging from 5,5 to 6,7, 10 mM sodium phosphate buffer was used in place of 10 mM tris buffer. At pH 6,7 both phosphate and tris buffers showed identical T_4 binding to matrix.

optimal around pH 6, with a second possible optimum at pH 7,5 (Fig. 9). This was in contrast to T_3 binding which showed a single optimum at pH 8 (Fig. 6).

The ability of iodothyronine analogues to inhibit T_3 binding to nuclear matrix was assessed. Curves of inhibition of [^{125}I]T_3 binding were constructed for two analogues, as shown by the representative experiment in Fig. 10. Maximal binding of [^{125}I]T_3 occurred at tracer levels and this progressively decreased as unlabelled T_3 or analogue was added. The relative affinity of the compounds for the matrix binding sites compared with T_3 were determined by comparing the concentration of ligand required to give a 50% depression of the tracer [^{125}I]T_3 binding. The molar concentration of T_4 required to produce a 50% depression was 6,1-fold greater than that of T_3. Similarly, rT_3 was about 65 times less effective than T_3 in causing a 50% depression. Therefore T_4 and rT_3 have relative binding affinities for matrix binding sites which are respectively 6,1- and 65-fold lower than that of T_3. The effects of a 200-fold molar excess of T_4 and T_3 on tracer [^{125}I]T_3 binding to matrix were also observed. It can be seen from Table II that the binding of [^{125}I]T_4 was inhibited to a similar degree by both the iodothyronines although T_3 did appear to be a better competitor.

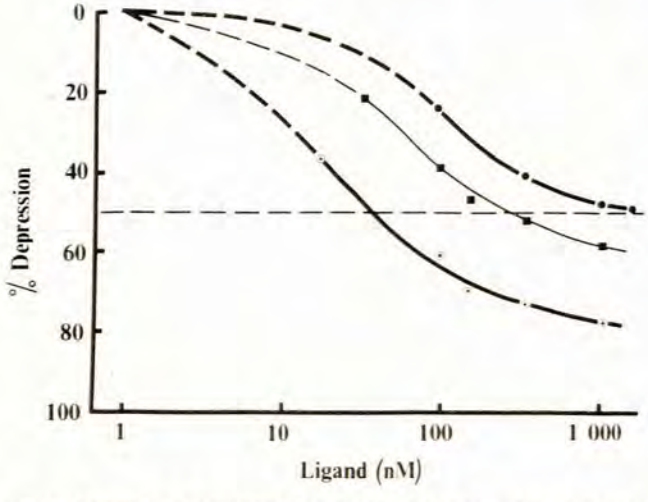

Fig. 10. Inhibition of T_3 binding to nuclear matrix by hormone analogues. [^{125}I]T_3, 1 nM, alone or with increasing concentrations of T_3 (⊙—⊙), T_4 (■—■) and rT_3 (●—●) were incubated with nuclear matrix (97 μg protein) as described under 'Materials and methods'.

TABLE II. INHIBITION OF [^{125}I]T_4 BINDING BY NON-RADIOACTIVE T_3 AND T_4

Hormone	[^{125}I]T_4 bound (fmol/mg matrix protein)
5 nM [^{125}I]T_4	149
5 nM [^{125}I]T_4 + 1 μM T_4	76
5 nM [^{125}I]T_4 + 1 μM T_3	52

Discussion

The results presented show that part of the salt-resistant T_3 binding to liver nuclei appears to be due to specific hormone binding to the insoluble matrix derived from these nuclei. Extensive DNase I and RNase digestion followed by extractions with 2M NaCl of purified rat liver nuclei after injection of [^{125}I]T_3 has revealed a class of specific T_3 binding sites which are associated with the liver nuclear matrix. Of the total T_3 bound to the liver nuclei from a single rat, 5% was bound to the insoluble matrix fraction. However, when the isolated matrix fraction was incubated directly with [^{125}I]T_3, the number of specific T_3 binding sites increased approximately 6-fold. Also, the incubation of intact nuclei with [^{125}I]T_3 revealed a 3-fold increase in the number of T_3 binding sites in the nuclear matrix. Approximately 50% of the total T_3 binding sites present in nuclear matrix could be saturated by the *in vitro* incubation of liver nuclei with T_3.

These results suggest that only a small proportion of the T_3 binding sites in nuclear matrix are labelled *in vivo* and that the majority can only be revealed after either nuclei or nuclear matrices are incubated directly with T_3. The *in vitro* labelling procedure may be capable of detecting both occupied and unoccupied T_3 binding sites, whereas labelling *in vivo* measures only unoccupied sites. These unoccupied sites may represent spare receptors. Similar findings for oestrogen binding to the matrix isolated from uterine nuclei have been reported by Barrack *et al.*[3,8]

The observed T_3 binding to the matrix fraction probably does not arise from entrapment of chromatin-bound T_3 owing to the incomplete extraction of the chromatin DNA, since isolated matrix, when further subjected to DNase digestion and extractions with 2M NaCl, retained its ability to bind T_3 to the same extent. Furthermore, when [^{125}I]T_3-labelled nuclei were fractionated in the presence of a 200-fold molar excess of unlabelled T_3, the total T_3 binding in nuclear matrix remained unchanged, indicating that matrix T_3 binding did not occur during isolation. It also seems unlikely that the matrix T_3 binding sites arise from absorption of chromatin T_3 receptor complexes which are released during the digestion and extraction procedures. Table I shows that the T_3 bound to the matrix was not proportional to the concentration of chromatin-associated T_3 receptors extracted from nuclei labelled with T_3 *in vivo* or *in vitro*. For the *in vivo* experiment, the matrix-associated T_3 made up 6% of the total soluble T_3 receptors, whereas the matrix-bound T_3 in the *in vitro* experiment was 20% of the total soluble T_3 receptors.

The binding characteristics of the nuclear matrix for iodothyronines were investigated *in vitro*. The nuclear matrix bound specifically T_3, T_4 and rT_3 but the avidity for T_4 and rT_3

was substantially lower, being 40% and 10% respectively that of T_3. Kinetic studies showed that the matrix binding sites were in equilibrium with the three iodothyronines at 3 - 6 hours at 20°C. Although T_3 binding also occurred at 4°C, equilibrium was only attained after approximately 18 hours. The binding sites were very stable at temperatures up to 20°C, but at 37°C less T_3 was bound and the amount bound decreased with time probably owing to the thermolability of the matrix binding sites. The rate of T_3 binding to matrix was slower than T_3 binding to 0,4M KCl soluble nuclear T_3 receptors; in the latter case, equilibrium binding was attained after 1 - 2 hours at temperatures between 0° and 20°C.[15,18] T_3 binding to matrix showed linearity over a wide range of matrix protein concentrations. A similar linearity has also been demonstrated with T_3 binding to nuclear extract[15,18] and isolated nuclei.[24] T_3 and T_4 probably bind to the same sites in the matrix, since each was able to inhibit the binding of the other.

Although both T_3 and T_4 binding to nuclear matrix was dependent on pH, the pH, at which optimal binding occurred was entirely different for the two compounds. The optimal binding for T_3 occurred at pH 8, whereas T_4 binding was optimal at about pH 6 (Figs 6 and 9). pH curves similar to those obtained for matrix binding have also been reported for T_3 and T_4 binding to 0,4M KCl nuclear extract.[25] Scatchard analysis demonstrated that the nuclear matrix has a single class of binding sites with a high affinity and a low capacity for T_3. The K_a for the matrix binding sites was $1{,}3 \times 10^9\ M^{-1}$ and the binding capacity of matrix isolated from 1 g liver was 76 fmol T_3. Competition experiments in which unlabelled T_3, T_4 and rT_3 were used to determine the relative displacement of tracer $[^{125}I]T_3$ showed that T_4 is bound 6-fold less tightly and rT_3 65-fold less tightly than T_3 to nuclear matrix. The relative affinities of the three iodothyronines studied correlate well with thyromimetic activities of these compounds.

Although there are several similarities in the properties of the chromatin-associated T_3 receptors and nuclear matrix binding sites for T_3, the matrix sites are probably not residual or adsorbed chromatin T_3 receptors, as already discussed above. One important difference, however, between the chromatin receptors and the nuclear matrix binding sites is that -SH groups are essential for T_3 binding to chromatin receptors,[18,26] whereas -SH groups do not appear to be involved in T_3 binding to the matrix.

The high-affinity, low-capacity characteristics of the T_3 binding sites associated with nuclear matrix and the correlation between their relative affinities for T_3, T_4 and rT_3 and the thyromimetic activities of these compounds suggest that matrix binding sites may function as hormonal receptors which regulate specific nuclear functions. The studies of Berezney and Coffey[27] and Pardoll *et al.*[6] suggest that the matrix may be involved in DNA replication. Furthermore, certain matrix proteins are phosphorylated and this phosphorylation precedes the onset of DNA synthesis.[7] Extensions of our *in vitro* studies on thyroid hormone binding to liver nuclear matrix could further advance the understanding of the mechanisms of action of the thyroid hormones at the molecular level.

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