DESOXYRIBONUCLEIC ACID COMPLEXES OF RARE EARTHS*

by

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E. HAMMARSTEN observed in 1924[8] that trivalent cations, *viz.*, lanthanum in the form of its nitrate and iron in the form of ferric sulfate, will form a precipitate when added in low concentrations to dilute solutions of the sodium salt of high-polymer desoxyribonucleic acid (STN), prepared essentially according to BANG's method. Additional experiments on the precipitation of STN by lanthanum salts were performed by E. HAMMARSTEN AND TEORELL[9], and by CASPERSSON, E. HAMMARSTEN AND H. HAMMARSTEN[2]. The maximum precipitation occurs in the pH-range from 2.5 to 4. It was shown that the lanthanum nucleinate complex has a very low solubility and that it contains 7.37% phosphorus.

Use was made of the reaction of lanthanum salts with nucleic acids in a number of subsequent investigations. Thus, lanthanum-containing reagents were employed for the fixation of nucleic acid structures in ultraviolet microscopy[3] and as electron stain of chromatin fibrils in electron microscopy[1]. Lanthanum salts have also been used in analytical studies on nucleic acids[6], and for the purification of desoxyribonucleic acid[5].

On chemical grounds, it is to be expected that other elements of the rare earth group, in their tripositive ionized state, will behave towards nucleic acid in a manner similar to that of lanthanum salts. Preliminary experiments in our laboratory, performed several years ago, showed that the salts of various rare earths (praseodymium, erbium, and yttrium) formed precipitates with sodium desoxyribonucleinate in aqueous solutions.

It was the object of the present investigation to obtain information on the composition and properties of the complexes formed between lanthanum and other rare earths with high-polymer desoxyribonucleic acid. This information was desired in connection with parallel investigations dealing with the distribution of lanthanum in the tissues of normal and tumor-bearing animals upon parenteral administration[10] and with the biological effects of rare earth compounds on mammalian cells[11] and microorganisms[14].

EXPERIMENTAL

Materials and methods

The rare earth compounds (lanthanum, praseodymium, samarium, and yttrium**) were obtained from Research Chemicals, Inc., Burbank, California, in the form of the oxides. The purity in all cases was over 99%. The chlorides of the rare earths were prepared from the oxides by means of hydrochloric acid.

Two preparations of high-polymer sodium desoxyribonucleinate (STN), obtained from calf

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** Yttrium, while not strictly one of the rare earth elements, possesses closely similar physical and chemical properties and is therefore usually grouped with them.

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thymus, were used in these experiments. The first was placed at our disposal by Professor R. SIGNER, Chemical Institute, University of Berne, Switzerland. It represents his Preparation No. VII, the isolation of which has been described by SCHWANDER AND SIGNER[13]. Its protein content is below 1 %. The viscosity behaviour of the material, as a function of the velocity gradient[13], indicates that it represents high-polymer STN which has undergone little or no degradation in the course of its preparation. The second STN preparation was a gift of Professor D. O. JORDAN, Department of Chemistry, University of Nottingham, England. Its designation is T 5/4, and it represents a second precipitation of the Preparation T 5/1, which has been described by GULLAND *et al.*[7]. The elementary composition of the material, as determined in Professor JORDAN's laboratory, is as follows: C, 37.7; H, 4.65; N, 16.3; P, 8.05 %. The sodium content of the first preparation (T 5/1), was 7 %[7]. Ultracentrifuge and diffusion studies on T 5/1 yielded a value for its molecular weight of $8.2 \cdot 10^5$.[4] It follows that this material, too, represents high-polymer sodium desoxyribonucleinate. Both preparations dissolved slowly in distilled water to yield clear and colourless, highly viscous solutions.

The spectrophotometric measurements were made with a Model DU Beckman quartz spectrophotometer.

The neodymium concentration in stock solutions of neodymium chloride was determined by measuring the optical density of the solutions at 522 mμ and employing a value of 0.030 for the absorption coefficient of neodymium chloride at 5218 A[12]. In the spectrophotometric experiments with STN the readings were made at 790 mμ where a steeper absorption maximum is situated. The absorption coefficient of $NdCl_3$ at this wave length was found to be 0.080, *i.e.* somewhat higher than indicated by the measurements of MOELLER AND BRANTLEY[12]. Since the absorption maximum of neodymium chloride at this wave length is very sharp, it was located by taking five readings in the vicinity of this wave length and using the maximum density value thus obtained.

OBSERVATIONS AND RESULTS

The rare earth complexes of desoxyribonucleic acid were prepared as follows:

100 mg of yttrium, samarium, or praseodymium chloride were dissolved in 10 ml of water, 5 ml of this solution were added to 20 ml of sodium desoxyribonucleinate solution containing approximately 0.8 mg STN per ml, and the resulting mixture was shaken for several minutes, and then filtered. The fibrous precipitate was washed with several hundred ml of water and then placed successively in ethanol-water mixtures of 25, 50, 75 and 96 % for five minutes at each concentration. Finally, it was immersed in ether for 10 minutes, then removed and allowed to dry in the air.

The complexes of neodymium and lanthanum with nucleic acid were prepared in a similar manner, except that larger volumes of the rare earth and nucleic acid solutions were used.

When moist, the nucleinates are tinged with the characteristic colour of the chloride of the rare earth salt employed, *e.g.*, the neodymium nucleinate is a pale pink, praseodymium nucleinate, a pale green, and the yttrium, samarium, and lanthanum nucleinates are colourless. When in the dry state, all of the preparations are white in appearance and have the texture of asbestos fibers.

With the exception of the lanthanum salt, pellets of the complexes, immersed in glycerol and transilluminated with a beam from a tungsten source, exhibited strong absorption bands in the visible region when viewed with a Zeiss hand spectroscope at room temperature. In the instance of the neodymium nucleinate complex, the bands were situated at 510–512, 520–525, and 570–590 mμ respectively, whereas the praseodymium complex exhibited absorption bands at 440–450, 465–470, and 475–480 mμ. The "didymium" complex, obtained by adding a mixture of neodymium and praseodymium chloride to a STN-solution, showed bands at 440–450, 465–470, 520–525, and 570–575 mμ. When the absorption spectra of the corresponding rare earth chlorides in aqueous solution were projected in a position adjacent to that of the spectra of the nucleinate complexes, no band shifts due to complex formation were detectable visually or photographically. However, the absorption bands of the nucleinates appeared to be

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somewhat broader and less sharply defined than the corresponding bands of the simple rare earth salts. The selective spectral absorption of rare earth nucleinates may lend itself to the development of a "staining" procedure for the microscopic study of chromatin and other nucleo-protein structures in cells and tissues.

Since samarium and europium exhibit fluorescence (*cf.*[15]) nucleinates of these metals were prepared in the usual manner and exposed to radiation from a mercury source, fitted with a filter which transmitted ultraviolet radiation of 366 mμ. No visible fluorescence was observed under these conditions.

Preliminary experiments to determine the solubility of neodymium nucleinate, were performed by treating weighed amounts of the complex with $NaOH–H_3BO_3$ buffer solutions, the pH of which ranged from 7.7 to 11.2. An additional tube containing the nucleinate and distilled water, was included as control. All tubes were shaken for twelve hours. The suspensions were then filtered and spectrophotometric determinations for neodymium and nucleic acid were made on the filtrate at 790 mμ and at 260 mμ respectively. In all instances, the concentration of neodymium present in solution was too low to be determined. The amount of nucleic acid in solution was found to be smallest in the water filtrate and greatest in the buffer of pH 9.0, indicating decomposition of the complex at this pH.

The elementary composition of an air-dried preparation of neodymium nucleinate, as found by micro-analysis (TIEDCKE) was: C, 26.14; H, 4.00; N, 11.21; P, 5.26; water, 7.15 and 7.08; ash, 20.76 and 20.67%. The corresponding values, after correcting for moisture, are: C, 28.1; H, 4.3; N, 12.1; P, 5.7; ash, 22.3%.

Several methods were tried to determine the content of rare earth and of nucleic acid in the neodymium nucleinate preparations. Hydrolysis of the compound in 1.0 *N* HCl, heated in a water bath for 45 minutes, left a very slight residue. Measurement of the optical density values at 790 mμ indicate that the complex contained 10.07% neodymium, corresponding to the combination of 1 mg neodymium with 8.93 mg DNA (average value obtained in four separate hydrolysis experiments).

If, on the other hand, the optical density of the hydrolysates was determined at 260 mμ, *i.e.*, at the nucleic acid absorption maximum, excessively high values for DNA-content were obtained. This may be due, in part, to the hydrolysis of the nucleic acid into free purine and pyrimidine bases, which are known to have higher extinction coefficients than STN at that wave-length, and to the absorption of neodymium salts in this region of the spectrum[12].

Attempts to determine the concentration of nucleic acid in the HCl digest by means of the Dische diphenylamine reaction were unsuccessful.

Because of the difficulties involved in the direct determination of the amount of nucleic acid in neodymium nucleinate preparations, an indirect method was tried which consisted in adding an excess of STN solution to a solution of neodymium chloride and shaking the mixture vigorously for several minutes to minimize the occlusion of STN in the precipitate. The excess STN in the filtrate was estimated colorimetrically by the Dische reaction at 620 mμ, employing a calibration curve prepared with the aid of a highly purified STN preparation placed at our disposal by Professor E. HAMMARSTEN, Karolinska Institute, Stockholm. The values thus obtained were lower than those obtained in the experiments described in the following section.

The characteristic absorption spectrum of neodymium chloride in aqueous solutions[12] made it possible to study the binding of neodymium chloride by sodium

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desoxyribonucleinate spectrophotometrically, by the following procedure: to an aqueous STN solution, usually containing approximately 0.8 mg STN/ml, was added an excess of neodymium chloride solution (0.5 to 1.5 ml containing 5.8 to 6.1 mg Nd/ml), the optical density of which had previously been determined at 790 mμ. This mixture was shaken for several minutes, the precipitate, consisting of neodymium nucleinate, was filtered off, and the amount of neodymium remaining in the supernatant solution was determined by spectrophotometry.

TABLE I

REACTION OF NEODYMIUM CHLORIDE WITH SODIUM DESOXYRIBONUCLEINATE (STN)

Expt.	*I* *DNA**	*II* *Nd*** *total*	*III* *Nd. residual (supernatant)*	*IV* *Nd**** *bound by STN*	*V* *DNA† bound per mg Nd*
No.	*mg*	*mg*	*mg*	*mg*	*mg*
1	6.98	3.313	2.487	0.826	8.45
2	6.98	6.626	5.894	0.732	9.54
3	6.98	9.393	8.510	0.883	7.90
4	7.58	2.906	1.993	0.913	8.30
5	7.58	2.906	1.119	0.787	9.63
6	7.68	2.906	1.981	0.925	8.30
7	7.69	2.906	1.981	0.925	8.30
8	7.69	2.906	1.981	0.925	8.30
				Average	8.55

* Amount of desoxyribonucleic acid contained in the STN used, calculated on the basis of 7.0% sodium content.

** Amount of neodymium in the neodymium chloride used.

*** Calculated as difference between the values in Column II and in Column III.

† Calculated by dividing the values in Column I by the values in Column IV.

It was found (see Table I) by this differential method that 1 mg neodymium binds, on the average, 8.55 mg of DNA. This would correspond to a neodymium content of the compound of 10.47%.

The micro-analysis (TIEDCKE) of an air-dried lanthanum desoxyribonucleinate preparation showed the following elementary composition: C, 26.84 and 26.68; H, 4.05 and 4.28; N, 6.74 and 6.82; P, 6.26 and 6.49; water, 7.24 and 7.37; ash, 25.70 and 25.51%. The subsequent analysis of another, carefully dried (at 110° C) lanthanum nucleinate preparation yielded an appreciably higher nitrogen content: N, 9.89 and 9.78%. Since it is known that substances of this type tend to form nitrogen-containing residues on combustion, the higher nitrogen values are probably more nearly correct. The P-content of this preparation was 6.52 and 6.39%. Correcting for moisture and using the higher nitrogen values yields the following elementary composition for the lanthanum nucleinate: C, 28.8; H, 4.5; N, 9.83; P, 6.67; ash, 27.6%.

The combination of lanthanum with desoxyribonucleic acid was also studied with the aid of radio-lanthanum (^{140}La), added as a tracer to stable lanthanum (^{139}La). From 1.0 to 5.0 ml of an STN solution containing 0.806 mg STN/ml were added to a constant volume, *viz.* 2.0 ml, of a lanthanum chloride solution containing 0.228 mg La/ml. The amount of lanthanum bound by the nucleic acid was determined by standard counting methods, measuring the amount of residual lanthanum in the supernatant solution as well as the lanthanum content of the lanthanum desoxyribonucleinate precipitate after

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acid hydrolysis. It was found that 1 mg lanthanum combines with 8.03 mg DNA (see Table II). On this basis, the lanthanum nucleinate complex contains 11.07% lanthanum.

TABLE II

REACTION OF LANTHANUM CHLORIDE WITH SODIUM DESOXYRIBONUCLEINATE, USING La^{140} AS TRACER

	I	II	III		IV	V
Expt.	*DNA* *	*La*** *total*	*La residual (supernatant)*	*La**** *bound by STN*	*La bound by STN*	*DNA† bound per mg La*
No.	*mg*	*mg*	*C.P.M.*	*C.P.M.*	*mg*	*mg*
1	0.750	0.504	13188	3103	0.0958	7.83
2	1.500	0.504	10490	6095	0.1850	8.11
3	2.250	0.504	7244	8929	0.2782	8.27
4	3.000	0.504	4260	12989	0.3795	7.91
5	3.750	0.504	1204	15784	0.4682	8.01
					Average	8.03

* Amount of desoxyribonucleic acid contained in the STN used, calculated on the basis of 7.0% sodium content.

** Amount of lanthanum in the lanthanum chloride used.

*** Determined in the hydrochloric acid hydrolysate of the lanthanum desoxyribonucleinate precipitate.

† Calculated by dividing the values of Column I by the values in Column V.

DISCUSSION

The chemical structure of the complexes formed between rare earth salts and desoxyribonucleic acid remains to be elucidated in detail. On the assumption that they represent salts which are analogous to sodium desoxyribonucleinate, the rare earth ions would be expected to combine with the acidic phosphate groups of the polynucleotide chain. Preliminary determinations with a flame photometer, using lithium as internal standard, indicate that in the compounds here prepared the sodium atoms are largely but not completely replaced by rare earth atoms. Thus, one of the La–DNA preparations appeared to contain approximately 0.3% Na while one of the Nd–DNA samples showed a residual sodium content of approximately 0.6%, as compared with a sodium content of 7% of the original STN-preparation. It is possible that the residual sodium content is due to the mechanical occlusion of some STN in the rare earth-DNA precipitates.

Considering the difference in valence and atomic weights between sodium and the rare earths, the La–DNA complex would be expected to contain approximately 14% lanthanum by weight, whereas our experiments with radio-lanthanum as tracer indicate a lanthanum content of about 11%, *i.e.*, 80% of the theoretical value. In the instance of the Nd–DNA complex, the corresponding figure, based on the spectrophotometric experiments using an excess of neodymium ions, amounts to 70% of the theoretical value. It is not possible to calculate the rare earth content of the solid compounds from the elementary analyses since the exact nature of the ash has not been determined.

Inasmuch as the phosphate groups of the polynucleotide chain are separated from each other by a linear distance of about 6 A along the backbone of the molecule, the combination of a single rare earth atom with three phosphate residues built into the *same* nucleotide chain would require the assumption of a tightly coiled polynucleotide helix with the rare earth atoms forming the core. Alternatively, the rare earth atoms might combine with phosphate groups of *adjacent* nucleotide chains, thus producing

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a network of nucleic acid threads cemented together by rare earth atoms. This might explain the insolubility of these complexes.

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SUMMARY

The reaction of lanthanum and other rare earth salts (neodymium, praseodymium, samarium, and yttrium) with the high-polymer sodium salt of desoxyribonucleic acid has been studied by spectrophotometric and tracer methods.

It was found that one part of lanthanum or neodymium combines with about 9 parts of desoxyribonucleic acid and that in this reaction a large fraction of the sodium content of the desoxyribonucleic is replaced by rare earth atoms. The elementary composition of the insoluble complexes produced in this manner has been determined. The tentative structure of the compounds formed between the rare earth elements and desoxyribonucleic acid has been briefly discussed.

RÉSUMÉ

La réaction des sels de terres rares (lanthane, néodyme, praséodyme, samarium et yttrium) avec le sel de sodium d'un acide désoxyribonucléique fortement polymérisé a été étudiée par spectrophotométrie et par les traceurs.

Une partie de lanthane ou de néodyme s'unit à environ 9 parties d'acide désoxyribonucléique et, au cours de cette réaction, une fraction importante du sodium est remplacée par des atomes de terre rare. La composition élémentaire des complexes insolubles formés de cette façon a été déterminée. La structure hypothétique des composés formés entre les terres rares et l'acide désoxyribonucléique est brièvement discutée.

ZUSAMMENFASSUNG

Die Reaktion von Lanthan und anderen Salzen der Seltenen Erden (Neodym, Praseodym, Samarium und Yttrium) mit dem hochpolymeren desoxyribonucleinsaurem Natrium wurde mit spektroskopischen und "Tracer"-Methoden untersucht.

Es wurde gefunden, dass sich ein Teil des Lanthans oder des Neodyms mit ungefähr 9 Teilen Desoxyribonucleinsäure verbindet und dass bei dieser Reaktion ein grosser Teil des Natriumgehaltes der Desoxyribonucleinsäure durch Seltene Erdatome ersetzt wird. Die Elementarzusammensetzung der auf diese Weise erzeugten unlöslichen Komplexe wurde bestimmt. Die versuchsweise angenommene Struktur der von den Seltenen Erdelementen und der Desoxyribonucleinsäure geformten Verbindungen wurde kurz besprochen.

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