beschreibt, sollte eine Auftragung von k gegen $[OH^{\ominus}]$ einen Ordinatenabschnitt ergeben, der eine Berechnung von k_{Im} erlaubt. Abb. 2 zeigt eine solche Auftragung und läßt erkennen, daß die Geraden durch den Nullpunkt gehen, d. h. daß das Imidazol an der Hydrolysereaktion nicht beteiligt ist, obwohl auf Grund der Struktur der Verbindungen eine intramolekulare Basenkatalyse durch den Imidazolrest begünstigt ist.

Zur Ermittlung der Aktivierungsparameter der Hydrolysereaktionen wurde die Temperaturabhängigkeit der Geschwindigkeitskonstanten gemessen. Die Ergebnisse dieser Messungen sind aus Tab. 2 zu entnehmen.

Verbindung	pH	$k \cdot 10^2$ [Min.$^{-1}$] 35°	50°	65°
1 a	10,0	1,83	9,68	25,0
1 b	10,0	1,38	6,00	19,8
1 c	10,5	1,36	4,19	9,95

Tab. 2. Temperaturabhängigkeit der Geschwindigkeitskonstanten k der Esterhydrolyse.

Die mit Hilfe dieser Werte errechneten Aktivierungsparameter der Hydrolyse sind in Tab. 3 aufgeführt. Wie man sieht, stimmen die ΔG^*-Werte für alle drei Verbindungen überein. Für Imidazol-4-carbonsäuremethylester findet man dagegen ΔH^*- und ΔS^*-Werte, die von denen der Imidazolpropionsäureester verschieden sind; darin kommt eine Kompensation von sterischen und Solvatationseffekten bei der Hydrolyse dieses Esters zum Ausdruck. Für die Imidazolpropionsäureester stimmen auch ΔH^* und ΔS^* trotz sehr unterschiedlicher Alkoholkomponente überein. Das bedeutet, daß bei beiden Verbindungen der gleiche Übergangszustand der Hydrolyse auftritt. Der fast doppelt so hohe negative ΔS^*-Wert für den Imidazol-4-carbonsäureester im Vergleich zu den Imidazol-4-propionsäureestern weist darauf hin, daß der aktivierte Komplex des Imidazol-4-carbonsäureesters weniger begünstigt ist, als der der Imidazol-4-propionsäureester.

Verbindung	ΔH^* [kcal/Mol]	ΔG^* [kcal/Mol]	ΔS^* [cal/Grad.Mol]
1 a	17,3	23,07	— 17,9
1 b	17,7	23,39	— 17,8
1 c	13,0	23,61	— 32,8

Tab. 3. Aktivierungsparameter der Esterhydrolyse.

Aus den Befunden ergibt sich, daß der Imidazolrest die Hydrolyse der untersuchten Ester weder in einer *inter*-molekularen noch *intra*-molekularen Katalyse beschleunigt. Serinester zeigen im allgemeinen eine Reaktivität, die man für Ester mit elektronenanziehenden Substituenten in der Alkoholkomponente erwartet [1]. Die relative Labilität des *O*-[Imidazol-4-(5)-propionyl]-*N*-Acetylserinamids ist dadurch hinreichend erklärt.

Vergleicht man diese Ergebnisse z. B. mit der durch Imidazol basenkatalysierten Hydrolyse des *N,O*-Diacetylserinamids [1], so ist u. a. zu berücksichtigen, daß diese Versuche in Imidazol-Puffern durchgeführt wurden, d. h. Imidazol gegenüber dem Ester in großem Überschuß vorhanden war.

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Formaldehyde Induced Changes of Heat-Denatured DNA

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While fractionating denatured DNA molecules from eukaryotic cells through hydroxyapatite (HA) column [1], it was seen that the elution profiles of DNA, denatured in absence and in presence of formaldehyde (hereafter called sample A and sample B respectively), were different under identical conditions of fractionation (Fig. 1 c and d). Elution profiles of sample A in presence and in absence of formaldehyde in the eluting buffer were also different (Fig. 1 c and b). In either case, when formaldehyde was present, greater quantity of denatured molecules appeared at a lower molarity of the eluting buffer. However, it is well known that the conformations of DNA in the two samples are different [1]. Except for a few native or native-like (double-stranded molecules with disordered regions) molecules, the bulk of the molecules in sample A were randomly coiled single polynucleotide strands as against extended single strands of sample B. In this communication, it is further shown that random coils of sample A are also extended when they are stored in presence of formaldehyde at room temperature. These changes in conformation satisfactorily explain the above differences in elution profiles.

From the osmotically hemolysed erythrocytes of an amphibian, toad *(Bufo melanostictus)*, DNA was isolated as described earlier [1]. The preparation of HA column material and the chromatography on that column were done by the step-wise elution technique as described by BERNARDI [2].

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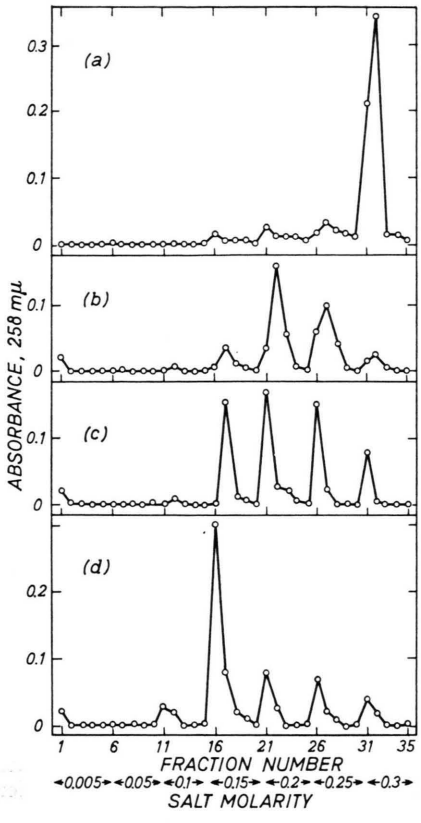

Fig. 1. Stepwise-elution of toad DNA (total 120 μg) loaded to 1.2 × 4 cm hydroxyapatite column, with phosphate buffer (pH 6.8) containing 1% formaldehyde (except for curve b). 3 ml fractions were collected. a) Native DNA. b) Sample A (20 μg/ml DNA heated at 100 °C for 10 min in 0.15 M NaCl and then rapidly chilled; relative absorbance 1.2) but eluted without formaldehyde. c) Sample A. d) Sample B (20 μg/ml DNA heated at 92 °C for 10 min in 0.15 M NaCl+1% formaldehyde and then rapidly chilled; relative absorbance 1.4).

Heat-denatured and rapidly cooled DNA (sample A) consisted mainly of randomly-coiled single strands except for about 5% native or native-like molecules (Fig. 2 *). However, the random coils were double-stranded over most of the regions by intrastrand folding and sparsely showed single-stranded regions. These structures eluted through HA without any detectable change in conformation in absence of formaldehyde [1]. But when the eluting buffer contained formaldehyde, the random coils were found to be very much uncoiled (Fig. 3). The native or native-like molecules, however, remained intact. In order to check, whether this change in conformation was due to the adsorption-desorption process itself or formaldehyde alone, the sample A was incubated in presence of formaldehyde (Fig. 6). The cubated sample when micrographed after about 4 hrs., the time for which the DNA sample of Fig. 3 was exposed to formaldehyde during and after the chromatography experiment before electron microscopy, uncoiling was noticed once again (Fig. 4). Conformational and spectral characteristics of the sample now became identical to those of sample B (Fig. 5). In this case also, the native or native-like molecules were unaffected.

As far as is known, the only property of the polynucleotide strands concerned with HA chromatography is their linear charge density [2], the single strands by virture of their lower linear charge density separate at a lower molarity of the eluting buffer than the double-stranded native DNA. On this basis as also from the evidence reported above, the discrepancies in the elution profiles can now be explained: (i) Since sample A is mostly in double-stranded form by intrastrand folding (Fig. 2) they should elute at a higher molarity than the extended single strands of sample B (Fig. 5) and that is what has been found by experiment. (ii) The hyperchromism of sample A when reacted with formaldehyde after cooling (Fig. 6) was observed previously [3]. It is further seen that even during the span of the chromatography experiment, formaldehyde can break open the random coils into open structures to an appreciable extent (Fig. 4). Since the single-stranded regions of the random coils now predominate over the double-stranded one, shift of the elution profile, in presence of formaldehyde, towards lower molarity is but expected.

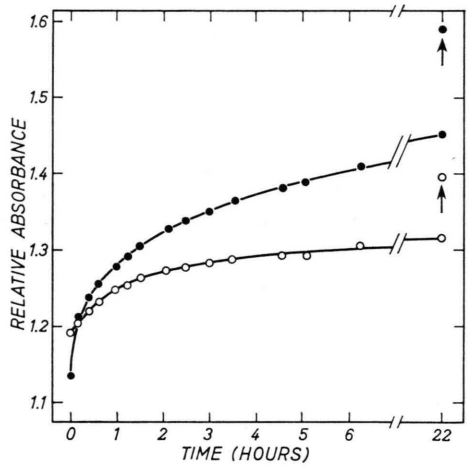

Fig. 6. Reaction of denatured DNA with formaldehyde. The DNA sample heated at 100 °C for 10 min in 0.15 M NaCl in 0.01 M phosphate buffer (pH 7.0), rapidly cooled and then reacted with 1% formaldehyde at 30 °C. Relative absorbances plotted against time of incubation in presence of formaldehyde. The arrow marked points give the relative absorbance when the incubed sample was further heated (100 °C for 10 min) and rapidly cooled. The curve at 258 mμ (—o—) follows denaturation and the one at 275 mμ (—●—) determines hydroxymethylation.

* Figs. 2—5 see table page 1318 a.

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The extension of the random coils in presence of formaldehyde at room temperature can be explained as follows. It is well known that formaldehyde reaction is only possible with free amino groups of polynucleotides and as such no reaction is expected with the nucleotide pairs of native DNA or the intrastrand pairs of denatured DNA (random coils). In other words, rupture of inter- or intrastrand H-bonds is a prerequisite for formaldehyde reaction. Hydrogen exchange studies, however, have suggested that even at low temperatures (well below melting region), the strands of DNA frequently open and rejoin [4]. The opening of the strands can thus set free the amino groups for formaldehyde reaction which, if it takes place, will prevent rejoining. With sufficient incubation, this process would lead to complete extension of the random coils as has been observed in the present experiments.

The following conclusions can be made. (i) The secondary structure of heat-denatured DNA is not altered by the adsorption-desorption process. (ii) Hyperchromism of formaldehyde reacted sample (Fig. 6) steadily rises up to about 2 hrs. and continues slowly thereafter. The time required in step-wise elution is also about 2 hrs. and, as such, enough change in the secondary structure of the single strands is possible even during fractionation. The shape of the elution profile, in presence of formaldehyde, is thus not only dependent upon the time that elapses during fractionation but also on the interval after which the absorbance measurements are done. (iii) Since the native or native-like molecules practically remain unaffected during the time the random coils are completely extended, it appears that the intrastrand binding of the random coils is not as strong as that existing between the twin strands of native molecules. (iv) The concept of frequent opening and closing of nucleotide pairs even under conditions of maximal stability is indirectly supported from the present study.

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Transcription of 4S RNA in *Limnaea* (mollusc) Embryos

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A general picture of the patterns of RNA synthesis during development has emerged in the last few years especially for sea-urchin [1, 2] and *Xenopus* [3]. Denis sums up the findings of Brown and his colleagues on *Xenopus* RNA with the help of a number of graphs so that transcription of different RNA fractions during developmental stages can be well visualised.

Such a study has now been carried out on *Limnaea* with the help of ion-agar electrophoresis. In the present note the transcriptoin pattern of 4 S RNA is being reported. Fertilized eggs before first cleavage, early and late morula, trochophores and veligers were investigated.

Following an earlier method [4], *Limnaea* eggs or embryos were kept in ^{32}P (phosphoric acid) solution in Tris buffer such that final pH was about 7.2. Normal development in this medium was checked at first. $25-100\ \mu C$ of radioactivity was used. RNA was extracted with the hot phenol method [4] and the radioactive RNA was mixed with the marker RNA from *E. coli*. Part of this RNA solution in 0.1 ml of 20% potassium acetate and 0.1 ml of 0.1 M sodium chloride was introduced in the groove cut in a gel of 1.25% ion agar (dissolved in citrate phosphate buffer of pH 8) layered over a microscope slide. Electro-phoretic runs of $60-75$ minutes using the same citrate phosphate buffer at a voltage of 350 and current 29 mA could generally separate the three bands of marker RNA. Tsanev [5] and Hadjiolov et al. [6] showed earlier that electrophoresis gives essentially the same results as density gradient and the heaviest RNA fraction lies nearest the origin. These facts as well as our comparative results with density gradient [7] strongly suggest that the markier bands are 23 S, 16 S and 4 S respectively, starting from origin.

We therefore estimated the newly synthesized RNA with the help of counts in different regions of the slide. The slide was stained and at the same time washed by putting it into a mixture of phosphate buffer and Toludine blue (final conc. of Tol. blue 0.1%). After the marker bands were visible, the bands and the regions in between the bands and pre 23 S and post 4 S regions were also cut out with shaving blades and washed overnight in phosphate buffer. The separation of bands is good only when a comparatively small

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