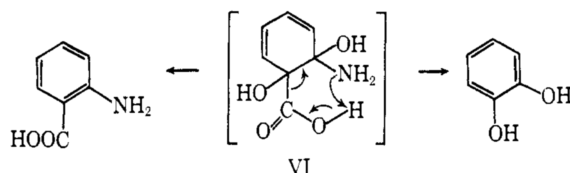
erated[26] by the Udenfriend reagent[27] or $O_2H\cdot$,[28] which are generally rather nonspecific,[29] cannot be ruled out, any more than can the "oxenoid" species postulated by Hamilton.[30]

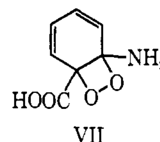
A quite different mechanism of oxygenation apparently occurs in microorganisms. A dioxygenase isolated[31] from *Pseudomonas* effects the incorporation of oxygen into anthranilic acid giving presumably the dihydro diol VI which spontaneously loses CO_2 and NH_3, to give catechol, possibly as shown. It has been suggested[32] on the basis of ^{18}O-labeling experiments that both atoms originate from molecular oxygen and that the cyclic peroxide VII is an intermediate in the formation of VI. The ring junction in VII must be *cis* and it follows therefore that the carboxyl group



VI

and the amino group in VI must also be *cis* to one another, thus facilitating the cyclic elimination shown.

(26) R. M. Acheson and C. M. Hazelwood, *Biochim. Biophys. Acta*, 42, 49 (1960).
(27) S. Udenfriend, C. T. Clark, J. Axelrod, and B. B. Brodie, *J. Biol. Chem.*, 208, 731 (1954).
(28) V. Ullrich and H. Staudinger, *Proc. U. S.-Japan Symp. Biol. Chem. Aspects Oxygenases*, 235 (1966).
(29) Reference 25, p 405.
(30) G. A. Hamilton, *J. Am. Chem. Soc.*, 86, 3391 (1964).
(31) H. Taniuchi, M. Hatanaka, S. Kuno, O. Hayaishi, M. Nakajima, and N. Kurinara, *J. Biol. Chem.*, 239, 2204 (1964).
(32) S. Kobayashi, S. Kuno, Itada, O. Hayaishi, S. Kozuka, and S. Oae, *Biochim. Biophys. Res. Commun.*, 16, 556 (1964).



VII

Experimental Section

Gas-liquid partition chromatography was carried out on a Glowall Model 310 chromatograph equipped with a 10-Mc ^{90}Sr argon ionization detector at 1000 v, using argon as a carrier gas. Mass spectra were measured on an A.E.I. MS-9 double-focussing mass spectrometer at 70 ev and on an LKB 9000 gas chromatograph–mass spectrometer, also at 70 ev. Accurate mass measurements were made by peak matching using perfluorotributylamine as a standard. Nuclear magnetic resonance spectra were measured in a Varian Associates HA-100 spectrometer using a frequency sweep.

The preparation of the mouse liver homogenate supernatant, the determination of its enzymatic activity, and the conversion of naphthalene to DHN-diol by this preparation have already been described.[1] The DHN-diol was purified by vacuum sublimation and recrystallization from benzene as colorless plates, mp 119–121°.

Enzymatic Formation of α-Naphthol. The α-naphthol formed in the enzymatic reaction was separated from the DHN-diol by thin layer chromatography on silica gel, and purified by glpc as its trimethylsilyl ether.

Nonenzymatic Formation of α-Naphthol. DHN-diol (*ca.* 0.1 mg) was dissolved in 2 *N* HCl (0.1 ml), and the solution was heated at 100° for 20 min. The aqueous solution was then extracted with ether (two 10-ml portions), and the ether was then evaporated off to give a mixture of α- and β-naphthols which were separated by glpc as above.

Preparation and Glpc of Trimethylsilyl Ethers. The mixture of naphthols obtained from 0.1 mg of DHN-diol was dissolved in bis(trimethylsilyl)acetamide (1 ml), and the mixture was allowed to stand at room temperature for 1 min, then chromatographed on (typically) a 6-ft column of 3% OV-1[33] at 120° with a carrier gas (He) pressure of 25 psi.

(33) Applied Science Laboratories, Inc., State College, Pa.

Molecular Sieve Entrapment. I. Entrapment of Deoxyribonucleic Acid by Cross-Linked Dextran (Sephadex)[1]

Robert Yolles

Contribution from Stanford Research Institute, Menlo Park, California 94025.
Received July 7, 1967

Abstract: Mechanical trapping of deoxyribonucleic acid (DNA) in a three-dimensional, hydrophilic matrix was accomplished by cross-linking dextran in the presence of single-stranded DNA. Diffusion of the trapped DNA molecules out of the three-dimensional matrix depends on the extent of cross-linking of the resin and on the time at which the DNA is added after initiation of the cross-linking reaction. Reactions of the resin-trapped DNA with nucleases of different molecular weights show that the ability of a nuclease to attack the trapped DNA depends on the size of the enzyme and on the degree of cross-linking of the resin.

Although clathrates or inclusion compounds are well known for small molecules,[2] and the diffusion of large and small molecules into molecular sieves has been amply described,[3] there is a scarcity of reports on the entrapment of large molecules into molecular sieves. This work deals with the *in situ* entrapment of macromolecules by molecular sieves. The feasibility of molecular sieve entrapment in a hydrophilic system was tested by trapping deoxyribonucleic acid (DNA) into Sephadex (a cross-linked dextran) by allowing the cross-linking reaction to take place in the presence of DNA, a macromolecule found suitable for this work because of its high molecular weight and well-characterized chemical properties.

(1) The author wishes to thank Dr. G. Freeman for consultive support, Mr. R. J. Jones for excellent technical assistance, and Dr. Z. Reyes for reading the manuscript prior to publication.
(2) F. Cramer, "Einschlussverbindungen," Springer-Verlag, Berlin, 1954.
(3) P. Flodin, "Dextran Gels and Their Application in Gel Filtration," Mejels Bokindustri, Halmstad, 1963.

Table I. Reactants and Reaction Times for Preparation of DNA–Sephadex[a]

Prep no.	H_2O, ml	5 N NaOH, ml	Epichlorohydrin, ml	DNA soln (or buffer) added, ml	DNA, μg	Time DNA or buffer added,[b] min	Total reaction time,[b] min	Water regain, g of H_2O/g of resin
I	0.86	0.60	0.34	0.20 Ha2°[c,d]	1.0	10	32	~10
II-A	0.60	0.60	0.40	0.20 AD7HaT[e]	150	5	50	~7.5
II-B	0.60	0.60	0.40	0.20 H_2O	...	5	50	~7.5
III-A	...	0.60	0.50	0.40 AD7HaT	300	0	55	4.5
III-B	...	0.60	0.50	0.40 AD7HaT	300	50	50	3.3
III-C	...	0.60	0.50	0.40 SSC[f]	...	60	60	3.3
IV-A	...	0.60	0.50	0.50 *E. coli*[g]	300	0	47	9.3
IV-B	...	0.60	0.50	0.50 Tris[h]	300	0	42	7.1
V	...	0.60	0.50	0.50 *E. coli*	300	0	60	6.2
VI	...	0.60	0.50	0.50 *E. coli*	300	0	250	3.0

[a] All reactions were carried out at 40 ± 1°. The amounts of the reactants used have been normalized to 1.0 g of dextran. [b] Elapsed times after start of reaction. [c] 7200 counts/min. [d] Ha2° = hamster secondary fibroblast cell culture DNA. [e] AD7HaT = adenovirus (type 7) induced hamster tumor DNA. [f] 1.0 × SSC. [g] *E. coli* K12 DNA. [h] 0.01 M Tris–HCl (pH 8.0).

Experimental Section

Materials. Dextran "40" ($\bar{M}$ = 40 × 10^3) and Sephadex G-10 were purchased from Pharmacia Corporation, Piscataway, New Market, N. J.; *E. coli* K12 DNA from General Biochemicals, Chagrin Falls, Ohio, and pancreatic DNase-I (E.C. 3.1.4.5) micrococcal nuclease from *Staphlycoccus aureus* (E.C. 3.1.4.7) from Worthington Biochemical Corporation, Freehold, N. J.

Absorption Measurements. Spectral analysis of the reaction of epichlorohydrin with the deoxyribonucleotides was made using a Cary 14 spectrophotometer. Other absorption measurements were made with a Beckman DU spectrophotometer.

Preparation of DNA. DNA was prepared from adenovirus (type 7) induced hamster tumor (Ad7HaT DNA) and from embryonic hamster secondary fibroblast cultures (Ha2° DNA).[4] The preparations are described elsewhere.[5] The DNA used in this work was estimated to have a molecular weight of 5–20 × 10^6 daltons.

Counting of Radioactive Samples. The activity of samples containing ^{3}H-DNA was measured using 2 ml of aqueous solution plus 8 ml of Bray's scintillation fluid.[6] Corrections were made for quenching, and the counts/min were normalized to those obtained in 0.01 × SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). Counts were made in a Nuclear-Chicago liquid scintillation counter.[7]

DNase-I Assay. A modification of the spectrophotometric assay of Kunitz[8] was employed using essentially the same reaction conditions as those described by Lakowski.[9] The final reaction mixture was 0.0045 M in Na_2EDTA (pH 8.0), 0.0075 M in $MnSO_4$, and 0.075 M in Tris–HCl (pH 8.0), and the concentration of heat-denatured *E. coli* K12 DNA was kept in the range 8–30 μg/ml. After addition of the enzyme, hyperchromism at 260 mμ (fractional increase in optical density) was measured in a Beckman DU spectrophotometer equipped with a cell compartment thermostated at 25 ± 1°.

Micrococcal Nuclease Assay. The assay used the conditions of Heins, *et al.*[10] The final reaction mixture was 0.01 M in $CaCl_2$ and 0.08 M in Tris–HCl (pH 8.0). Measurements of hyperchromism were made as described for the DNase-I assay.

Preparation of DNA–Sephadex. The method of preparation was similar to that of Flodin.[3] The resins were prepared in a reaction vessel thermostated at 40 ± 1° and equipped with a motor-driven stirrer. The amounts of reactants used and the reaction times are given in Table I. The procedure adopted was as follows. The dextran was wetted with the aqueous solution (water, buffer, or DNA in buffer), after which the NaOH was added. This was followed by the epichlorohydrin (at zero time) and the other solutions as indicated. After the reaction was complete the resin was neutralized with dilute HCl and disintegrated by mincing, pushing through a stainless-steel screen, or using a blender. It was then washed several times with water. For DNA–Sephadex-II-A and -B the neutralizing solutions were not discarded but were used with the resin for the 75° extraction experiment. Some resin preparations were dehydrated by washing with progressively increasing concentrations of ethanol (in water) prior to overnight drying *in vacuo* at 50–70°.

The water regain[3] (1 g of H_2O taken up per g of dry resin) was measured by soaking a weighed amount of dry resin in water overnight, drawing off the excess water by suction through a sintered-glass funnel, and weighing the wet resin.

Results

Evidence That DNA Is Not Altered by Cross-Linking Reaction. In order to assess the properties of DNA as a molecular species after trapping, it was necessary to establish that the cross-linking reaction did not chemically alter the DNA. For this purpose a control reaction was carried out using DNA monomeric constituents, the deoxyribonucleotide 5′-monophosphates (5′-dAMP, 5′-dCMP, 5′-dGMP, and 5′-dTMP), with epichlorohydrin. The ultraviolet spectral properties of the nucleotides taken through the control reaction were examined and compared with literature values (Table II). The good agreement between the experimental values and those of the literature suggests that, at most, only a small fraction of the DNA bases react with epichlorohydrin under the experimental conditions. Minor differences in the spectral ratios may be due to the reaction at the 3′-hydroxyl of the nucleotide, since the normality of the base used in the reaction (1.5 N) is sufficient to ionize the 3′-hydroxyl and render it susceptible to attack by the cross-linking agent. In this work the acid spectra are considered less reliable because some precipitation or aggregation at low pH of an ultraviolet-absorbing, epichlorohydrin by-product occurred. In the case of 5′-dAMP, aggregation in acidic solution is known to lead to a long-wavelength tailing in the absorption spectrum.[11]

Evidence That the DNA Is Not Covalently Attached to Resin. The possibility that the DNA may be attached covalently to the cross-linked resin is ruled out by experiments in which the DNA was added only 5 or 10 min after start of the cross-linking reaction. A

(4) The author expresses his thanks to Dr. I. Sultanian and Mr. L. Hooser for a supply of AD7HaT and Ha2° cells.

(5) R. S. Yolles and G. Freeman, *Biochim. Biophys. Acta*, **138**, 506 (1967).

(6) G. A. Bray, *Anal. Biochem.*, **1**, 279 (1960).

(7) The author thanks Dr. G. LePage for use of the scintillation counter.

(8) M. Kunitz, *J. Gen. Physiol.*, **33**, 349 (1950).

(9) M. Lakowski in "Procedures in Nucleic Acid Research," G. L. Cantoni and D. R. Davies, Ed., Harper and Row, Publishers, New York, N. Y., 1965, p 85.

(10) J. N. Heins, H. Taniuchi, and C. B. Anfinsen in "Procedures in Nucleic Acid Research," G. L. Cantoni and D. R. Davies, Ed., Harper and Row, Publishers, New York, N. Y., 1966, p 79.

(11) S. Basu and J. H. Greist, *J. Chim. Phys.*, 60, 407 (1963).

Table II. Ultraviolet Properties of 5'-Deoxyribonucleotide Monophosphates Treated with Epichlorohydrin[a]

	A_{250}/A_{260}		A_{280}/A_{260}		A_{290}/A_{260}		λ_{max}, mμ		λ_{min}, mμ	
	Exptl	Lit.[c]	Exptl	Lit.[c]	Exptl	Lit.[c]	Exptl	Lit.[c]	Exptl	Lit.[c]
pH 2.0[b]										
dAMP	0.85	0.81	0.29	0.22	0.08	0.03	258	258	231	228
dCMP	0.46	0.44	2.02	2.10	1.39	1.58	279	280	241	239
dGMP	1.12	1.01	0.68	0.71	0.42	0.52	253	255	231	227
dTMP	0.81	0.63	0.72	0.71	0.31	0.25	266	267	240	233
pH 12.0										
dAMP	0.77	0.79	0.18	0.15	0.02	0.00	259	260	229	226
dCMP	0.82	0.81	0.94	0.97	0.28	0.30	271	271	250	250
dGMP	0.89	0.91	0.57	0.62	0.13	0.12	259	262	230	230
dTMP	0.68	0.67	0.67	0.67	0.20	0.18	268	267	243	243

[a] Each reaction mixture contained 3.3 ml of an aqueous solution which was 150 μg/ml with respect to the nucleotide, and 1.5 *N* in NaOH. To each solution was added 1.7 ml of epichlorohydrin, which is only very slightly soluble in the aqueous reaction mixture. After intermittent agitation for 75 min at room temperature, the aqueous layers were removed, extracted with $CHCl_3$–octanol-1 (24:1 v/v), diluted with H_2O, and titrated with HCl to adjust the pH. A control reaction mixture contained 0.01 *N* NaOH in place of the nucleotide solution. Spectra were measured in a Cary Model 14 spectrophotometer. [b] Except for dGMP which was measured at pH 1.0. [c] Schwartz Biochemical Research Catalog, 1966, and references therein.

quantitative recovery of the DNA was obtained even though the reaction period was nearly as long (*i.e.*, about 1 hr) as that used when the DNA was added at the beginning of the cross-linking reactions and in which it was effectively trapped. Compare, for example, the results shown in Figure 1, in which the DNA was added at zero time, and only 23% of it was recovered from the resin, with the data shown in Figure 2, in which the DNA was added 5 min after zero time and 100% was recovered, or the data shown in Figure 3, in which the DNA was added 10 min after zero time and a recovery of 64% (fractions 1–45) was obtained. The recovery in this latter case would presumably have been greater if additional fractions had been collected. Furthermore, experiments performed with *E. coli* K12 DNA added at zero time to resin preparations IV-A, V, and VI (Figures 4 and 5) showed that no measurable amount of DNA was released even during incubation periods of several days. These latter experiments were carried out at room temperature and were part of the enzyme studies discussed below.

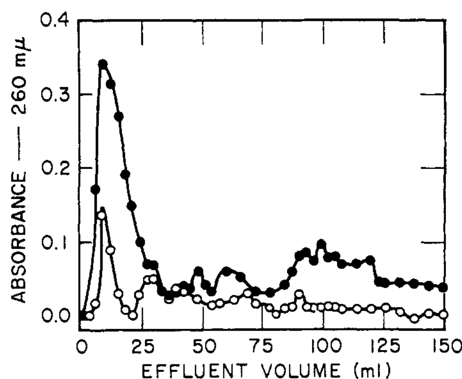


Figure 1. The elution of DNA from DNA-Sephadex-III to which *E. coli* K12 DNA (10.6 OD units) was added before, O, or after, ●, the cross-linking reaction; 2.1 and 11.0 OD units, respectively, were recovered from each resin. Optical density values corresponding to a control resin (buffer added in place of DNA) have been subtracted from the points shown. After preparation of the resins they were placed directly into the columns (there was no prior washing or attempt to extract reaction by-products) and elution was carried out at 60° using 2 × SSC for 81 ml followed by elution at 75° using 0.01 × SSC for 79 ml. The column size was 4.5–6.0 × 1.15 cm and the flow rate varied from 20 to 40 ml/hr.

Outward Diffusion of Trapped DNA. Diffusion of DNA added before or after the start of the cross-linking reaction is shown in Figure 1, which depicts experiments in which a column chromatographic technique was used and the amount of eluted DNA was measured by ultraviolet absorption. Elution was first carried out under conditions in which single-stranded *E. coli* K12 DNA substantially renatures to the double helix.[12] The elution conditions were then changed to those known to heat denature *E. coli* DNA.[12] Although no additional DNA was eluted from the resin in which the DNA was added at the start of the reaction, an appreciable amount was eluted from the resin in which the DNA was added after initiation of the cross-linking reaction. These results indicate that the double helix structure itself does not materially prevent the outward diffusion of DNA from the resin.

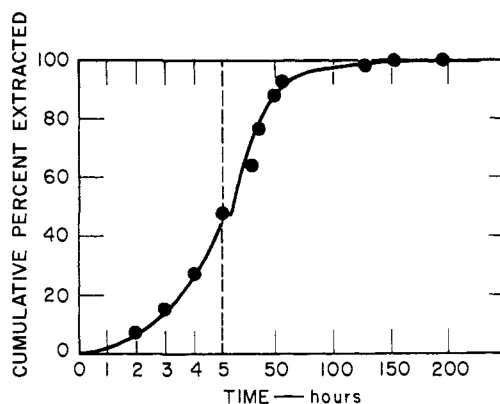


Figure 2. The extraction of DNA from DNA-Sephadex-II. Five minutes after start of the cross-linking reaction 1.0 ml (5.1 OD units, corresponding to 100%) of AD7HaT DNA was added to reaction mixture (1.0 ml of water was added to the control). After 50 min, the resin was neutralized, disintegrated, and placed into tubes incubated at 75°. At the times indicated the mixture was centrifuged, the 3-ml supernatant was removed, the optical density was determined, and 3 ml of fresh 0.1 × SSC was added to each tube.

(12) J. Marmur and P. Doty, *J. Mol. Biol.*, 3, 585 (1961).

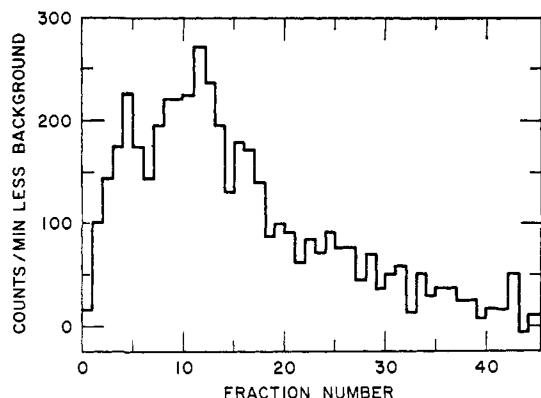


Figure 3. Elution of ^{3}H-Ha2° DNA from DNA–Sephadex-I. A total of 7200 counts/min of DNA was added to the resin preparation of which 4600 counts/min (64%) was recovered from fractions 1–45 (each fraction was 7.3 ml). The column was thermostated at 75° and elution was carried out using 0.01 × SSC. The column dimensions were 2.54 × 15–20 cm. The flow rate varied from 10 to 30 ml/hr.

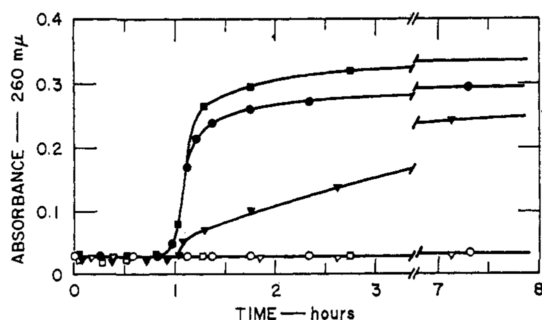


Figure 4. Action of micrococcal nuclease on DNA–Sephadex-IV, -V, and -VI, having water-regain values of 3.0, ▼; 6.2, ●; and 9.3, ■, respectively. Control experiments, in which buffer was added in place of the enzyme solution, are indicated by the corresponding open symbols. The enzyme incubations were carried out as follows: to 0.20 g of dry resin was added 0.60 ml of 0.1 M $CaCl_2$, 4.8 ml of 0.1 M Tris-HCl (pH 8.0), and, at zero time, 0.4 ml of 0.8 μg/ml micrococcal nuclease in 0.1 M Tris-HCl (pH 8.0). After 53 min, 0.2 ml of 8.0 μg/ml micrococcal nuclease was added. The reaction conditions are those used for the enzyme assay described in the caption for Figure 6.

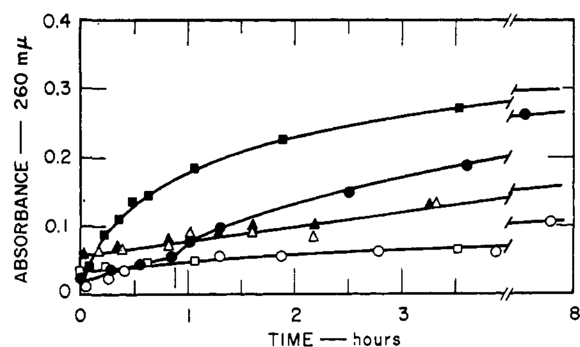


Figure 5. Action of DNase-I on DNA–Sephadex-IV, -V, and -VI, having water-regain values of 3.0, ▼; 6.2, ●; and 9.3, ■, respectively. Control experiments, in which water was added in place of the enzyme solution, are indicated by the corresponding open symbols. The enzyme incubations were carried out as follows: to 0.20 g of dry resin was added 0.60 ml of 0.045 M Na_2EDTA (pH 8.0), 0.60 ml of 0.075 M $MnSO_4$, 4.4 ml of 0.1 M Tris-HCl (pH 8.0), and, at zero time, 0.20 ml of 25 μg/ml DNase-I in H_2O. After 53 min, 1.0 ml of additional 25 μg/ml DNase-I was added. The reaction conditions are those used for the DNase-I indicated in Figure 6. The amounts of DNase-I added correspond in activity to the amounts used for the corresponding experiments with micrococcal nuclease (Figure 4) but the latter enzyme is inhibited by and/or adsorbed onto the resin particles and glass reaction vessel (see text).

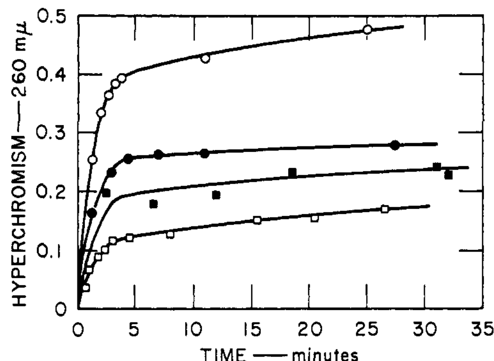


Figure 6. Assay of micrococcal nuclease and deoxyribonuclease-I in the presence or absence of Sephadex. The micrococcal nuclease assay was performed in the presence, ●, or absence, ○, of G-10 Sephadex (water regain = 1.0). The DNase assay was performed in the presence, ■, or absence, □, of Sephadex having a water regain of 9.0. For the micrococcal nuclease assay each solution was 0.01 M in $CaCl_2$, 0.075 M in Tris-HCl (pH 8.0), 267 mμg/ml with respect to micrococcal nuclease, and 29 μg/ml with respect to denatured *E. coli* K12 DNA. For the DNase-I assay each solution was 0.0045 M in EDTA (pH 8.0), 0.0075 M in $MnSO_4$, 0.075 M in Tris-HCl (pH 8.0), 400 mμg/ml with respect to DNase-I, and 7.5 ± 0.5 μg/ml with respect to heat-denatured *E. coli* K12 DNA. Note that in the presence of the resin the concentration of DNA in the void is initially greater since it is initially excluded from the resin.

Although it has not been the intention of this work to measure precisely the outward diffusion of DNA trapped within the molecular sieve, the data shown in Figures 2 and 3 give some indication as to rate of elution under certain conditions. In both experiments DNA was added to the resin reaction mixture after the cross-linking reaction was begun. The data of Figure 2 correspond to an elution in which about 20 hr was required to extract 65% of the DNA. The data corresponding to Figure 3 indicate that 10–30 hr was required to extract 65% of the DNA. Because adding the DNA at the beginning of the reaction very greatly diminishes its ability to diffuse out of the matrix (Figure 1 compared with Figures 2 and 3), it is clear that the outward diffusion of the macromolecule is controlled in part by the time at which the macromolecule is added after initiation of the cross-linking reaction. Furthermore, it is clear that the final degree of cross-linking attained by the resin affects the outward diffusion of the trapped species.

Permeability of DNA–Sephadex to Nucleases. Experiments were carried out to test the permeability of the DNA–resin to enzymes of two different sizes. The enzymes employed were micrococcal nuclease, molecular weight 11–12 × 10^6 daltons,[13] and deoxyribonuclease-I, molecular weight 63 × 10^6 daltons.[8,14,15] These enzymes were added to DNA–Sephadex preparations having water-regain values of 3.0, 6.2, and 9.3 as shown in Figures 4 and 5. The amounts of enzyme added had equal activities under the assay conditions described (Figure 6). Micrococcal nuclease, however, is inhibited by glass; this effect is attributed to adsorption of the enzyme onto the

(13) C. B. Anfinsen, M. K. Rumley, and H. Taniuchi, *Acta Chem. Scand.*, 17, S270 (1963).
(14) M. Kunitz, *J. Gen. Physiol.*, 33, 363 (1950).
(15) C. L. Smith, *Arch. Biochem. Biophys.*, 45, 83 (1953).

walls of the reaction vessel.[10] A similar adsorption onto the surface of the resin particles may occur. The difference in activity, illustrated in Figure 6, accounts for the lack of micronuclease activity when initially added to DNA–Sephadex (Figure 4). Hence additional enzyme (five times the original amount) was added after 52 min to each resin preparation. The experiments using deoxyribonuclease-I (Figure 5) were performed in the same way so that an exact comparison could be made.

The results of the experiments using Sephadex-trapped DNA (Figures 4 and 5) indicate that DNA–Sephadex-IV (M_e = 58,000 daltons)[16] is easily permeated by both enzymes. DNA–Sephadex-V (M_e = 23,000 daltons) shows greatly reduced permeability to deoxyribonuclease-I, but only slightly reduced permeability to micrococcal nuclease. For DNA–Sephadex-VI (M_e = 5000 daltons) no detectable activity of deoxyribonuclease-I was observed. Micrococcal nuclease, although somewhat reduced in activity, was still active in degrading trapped DNA.

Discussion

The results obtained for the DNA–Sephadex complexes strongly suggest that the biopolymer has been mechanically trapped by *in situ* cross-linking of the dextran, and that by varying the reaction parameters the degree of entrapment can be varied. In principle one should be able to carry out similar reactions with a variety of large molecules. Since numerous methods are known for producing a three-dimensional matrix (either hydrophilic or hydrophobic), it should be possible to select conditions that do not affect the trapped species. It should be possible, for example, to trap a large catalyst (*e.g.*, an enzyme) which could selectively attack only those substrates small enough to diffuse into the resin. This type of experiment would be the reverse of that described in this work, in which the substrate was trapped and enzymes were selectively allowed to diffuse into the resin. An interesting application of this principle would be the removal of molecules whose size is less than M_e, since in a heterodisperse mixture (with respect to molecular weight) smaller molecules could enter the gel, be degraded to very small size, and thus be easily separated from the mixture.

In these studies a specific example of molecular sieve entrapment has been demonstrated, *viz.*, the assay of nucleases of differing molecular weight. However, other applications of the method should be possible. Among these are the slow, controlled release of vaccines, enzymes, drugs, catalysts, or large molecules into the surrounding environment. The basic consideration is that one or more of the reactants be sufficiently greater in size than the others so that the differentially permeable gel can bring about selectivity in the reaction.

(16) The exclusion limits of the DNA–Sephadex preparations were estimated by use of the following empirical relation, which was derived from product literature of Pharmacia Corporation: $\log M_e = 2.14 \log R + 2.68$, where M_e is the exclusion limit in daltons and R is the water regain.

Communications to the Editor

A Dibenzohomotropylium Ion[1]

Sir:

A considerable number of monohomotropylium-type cations are now recognized.[2] Even the 1-hydroxy-substituted species[2g] is decidedly homoaromatic and is capable of sustaining a large induced ring current. In exploring this phenomenon further, it seemed to us that a homocounterpart of the 2,3,6,7-dibenzotropylium ion[3] (II) would be instructive from the viewpoint of homoaromaticity[2f] since the two benzene rings in I and II dampen considerable the gain in ΔE_π due to cyclic electron delocalization attending formation of a tropylium species.[3b] An additional interest in dibenzohomotropyl derivatives VIII was the comparison of any rate-enhancing effect of the cyclopropane ring in VIII with that of the spirocyclopropyl group in the ionization[4] of IV to the anthrylethyl bridged ion[4b] V.

Cyclopropanation of the olefinic group in dibenzotropyl derivatives proved difficult but was finally accomplished by the action of $C_6H_5HgCBr_3$ on the dibenzotropone. Reduction of the dibromo ketone[5] VII-ketone, mp 177–178°, gave rise to the *cis* alcohol[5] VII-OH, mp 160–161°, which was debrominated by way of the tetrahydropyranyl ether with the aid of *n*-Bu_3SnH. The debrominated *cis*-VIII-OH,[5] mp 143–144°, equilibrated with the *trans*-VIII-OH,[5] mp 135–137°, to an 80.8% *cis*–19.2% *trans* equilibrium composition in acidified 80% aqueous dioxane at 75°. Similarly, the equilibrium between *cis*-VIII-OMe,[5] mp 93.5–94°, and *trans*-VIII-OMe,[5] mp 56–58°, was 56.4% *cis*–43.6% *trans* in MeOH at 65°, and that between *cis*-VIII-OAc,[5] mp 150–151°, and *trans*-VIII-OAc,[5] mp 141–142°, was 46.5% *cis*–53.5% *trans* in Ac_2O at 100°.

Configurations and conformations were assigned to the VIII epimers on the basis of nmr studies.[6] As

(1) Research supported by the National Science Foundation.

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(5) The indicated compounds had satisfactory elemental analyses and appropriate infrared and nmr spectra.

(6) F. A. L. Anet, M. Brown, R. F. Childs, and S. Winstein, unpublished work to be reported separately.