Partial Resolution of DL-Adenosine by Template Synthesis

Abstract. *D-Adenosine-5′-phosphorimidazolide reacts very much more rapidly with D-adenosine than with L-adenosine on a poly-D-uridylic acid template. This permits the partial resolution of DL-adenosine. These experiments suggest that segregation of D- and L-nucleotides may have occurred at an early stage in biochemical evolution.*

We have shown that poly U_D directs specifically the condensation of activated pA_D with A_D (*1–3*). Here we investigate the effect of optical configuration on the reaction. Our results are relevant to emergence of optical activity in the nucleic acids.

We have studied the condensation of A_D and A_L with $ImpA_D$ on a poly U_D template. Two reaction mixtures were prepared at 0°C. Each contained D-adenosine-5′-phosphorimidazolide-8-C^{14} (about 0.2 μc/μmole), 0.003*M*; poly U_D, 0.05*M* in uridylate residues; imidazole, 0.2*M*; NaCl, 0.2*M*; $MgCl_2$, 0.075*M*. In addition, one solution contained A_D (0.022*M*) and the other an equal amount of A_L. Control solutions were prepared with the poly U_D omitted. The solutions were adjusted to *p*H 7.5 with 2*M* HCl.

After 14 days, the *p*H of the solutions had not changed. Samples were withdrawn and analyzed by methods described (*1*); NH_2pA_D which contaminated the $ImpA_D$ starting material was separated from ApA by electrophoresis at *p*H 7.5. The amounts of products identified are given in Table 1. The proportions of the different isomers of A_DpA_D were not determined because they have been reported for a very similar experiment (*2*). The chromatographic behavior of the material assumed to be A_LpA_D was very similar to that of A_DpA_D. The ultraviolet spectrum was characteristic of A. Degradation was almost complete in 1*M* KOH after 15 hours at 37°C, giving radioactive A and nonradioactive Ap. We conclude that the A_LpA_D contained little, if any, of the 5′ → 5′ isomer.

In these reactions, 13 times more A_DpA_D than A_LpA_D was formed. In the control experiments without poly U_D only very small amounts of the phosphodiesters were obtained. Here too, A_D is favored, but to a lesser extent.

The other radioactive products must have arisen from the self-condensation of the $ImpA_D$. They were identified by chromatography against authentic samples. The pA_DpA_D is formed in higher yield in the template experiment with A_L, because the imidazolide is not removed in the template reaction with A_D. This is confirmed by the more rapid disappearance of $ImpA_D$ in the tube containing poly U_D and A_D than in any other tube.

To study the competition of A_D with A_L on a poly U_D template, two reaction mixtures (0.5 ml) were prepared. Each contained: $ImpA_D$, 0.012*M*; poly U_D, 0.05*M* in uridylate residues; imidazole, 0.2*M*; NaCl, 0.2*M*; $MgCl_2$, 0.075*M*. In addition, our first reaction was 0.006*M* in D-adenosine-8-C^{14} (0.26 μc/μmole), and 0.006*M* in A_L; these amounts are sufficient, together with the $ImpA_D$, to fill the available A sites in the triple helix. Our second reaction was 0.012*M* in each of these nucleosides. The solutions were kept at *p*H 7.6 ± 0.1 and 0°C for 14 days. Yields of product, based on the total radioactivity, are given in Table 2.

The absolute yield of A_DpA_D is almost the same in experiments 1 and 2. In experiment 1, we know that almost all of the A_D, A_L, and $ImpA_D$ are in the triple helical structure. If A_L was displaced from the helix by the excess of A_D in experiment 2 we would anticipate an increased yield of A_DpA_D. Thus our experiments show that A_L competes effectively for space on the template. However, we cannot say whether it displaces A_D, $ImpA_D$, or both.

The specific activity of the A recovered from experiment 1 was found to be 7920 count/min per optical density unit. The specific activity of the starting material was 13,000 count/min per optical density unit. Therefore, the proportion of A_L had increased from 50 to 69 percent. This ratio was confirmed by treatment of the A with adenosine deaminase (E.C. 3.5.4.4). Samples containing varying ratios of A_D to A_L were prepared, and the change in the ultraviolet absorption at 265 nm was measured after treatment with the enzyme. The same procedure applied to the unknown mixture showed that it contained 68 percent of A_L. If no A_L had been incorporated into the ApA and ApApA the unreacted A would have contained 69 percent of A_L (Table 2). If one part of A_L had been incorporated for 13 parts of A_D, as suggested by our previous experiments, 68 percent of A_L would have been present.

Direct measurement of the optical rotatory dispersion spectrum of the recovered adenosine showed that it had roughly the expected optical activity. However, we could not estimate the extent of resolution accurately on account of the low specific rotation of adenosine.

The fact that all proteins are composed of L-amino acids and all nucleic acids of D-ribonucleotides or D-deoxyribonucleotides has occasioned extended discussion. It seems almost certain that the success of the contemporary biochemical system rather than the completely enantiomeric one was a matter of chance. It is still not clear whether a system of D-amino acids and D-nucleotides could have evolved as easily as ours and competed successfully with it, nor do we know why all amino acids in proteins have the same optical configuration. However, while the normal double-helical structure of the nucleic acids can be built either with D-nucleotides or L-nucleotides, no simple, regular structure can be made from a pair of chains each of which contains both D- and L-monomers. It is just possible that a related structure could be built from one D-chain and one L-chain. Thus on theoretical grounds it is expected that replicating nucleic acids must be made up of optically homogeneous chains, probably having the same handedness (*4*).

Our results support and extend these theoretical arguments. Poly U_D directs an efficient synthesis of A_DpA_D from A_D and $ImpA_D$ but has little effect on the corresponding reaction with A_L. Thus, as we have shown, it helps to bring about the resolution of racemic

Table 1. Percentage distribution of radioactivity from the condensation of D- and L-adenosine with D-adenosine-5′-phosphorimidazolide-8-C^{14}.

Components	ApA_D	pA_DpA_D	A_DppA_D	pA_D	$ImpA_D$	NH_2pA_D
A_D + $ImpA_D$	0.9	< 0.1	2.2	41.4	51.5	4.0
A_L + $ImpA_D$	0.3	< 0.1	2.0	41.2	52.5	4.0
A_D + $ImpA_D$ + polyU_D	37.8	1.0	2.9	34.7	18.9	4.7
A_L + $ImpA_D$ + poly U_D	2.9	3.5	3.1	37.8	44.2	5.7

Table 2. Percentage distribution of radioactivity after reaction of D-adenosine-5′-phosphorimidazolide with a racemic mixture of D-adenosine-8-C^{14} and L-adenosine.

Reaction	A_D	A_DpA_D	$A_DpA_DpA_D$
1	45	51	4
2	73	26	1.1

adenosine under appropriate conditions. Oligoadenylates formed on a poly U_D or poly U_L template from DL-adenosine derivatives should, therefore, be optically homogeneous. Although we have not excluded the formation of L-chains on a D-template, we think this less likely than the formation of D-chains.

The work of Huang and Ts'O (*5*) shows that A_D and A_L form triple helices with poly U_D, which are of comparable stability. The specificity of the condensation reaction probably has its origin in the different relative orientations of 5′-phosphate and 2′-hydroxyl groups for D- D- and D-L- neighbors, but it could also be due to the segregation of enantiomers on the template.

Our results suggest that, when template-directed synthesis is predominant, optically homogeneous oligonucleotides can, in principle, be formed from initially racemic mixtures. If the formation of a "nucleus" for effective synthesis is highly improbable, then all templates in a given microenvironment would arise from a single ancestor and, in all probability, have the same configuration. Otherwise equal numbers of D- and L-chains would be expected. The important point for discussions of the origin of life is that optically homogeneous chains have a selective advantage whenever template synthesis occurs.

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References and Notes

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3. Abbreviations: polyU$_D$, poly-D-uridylate; A$_D$, D-adenosine; A$_L$, L-adenosine; A, adenosine (optical isomer not specified); pA$_D$, D-adenosine-5′-phosphate; ImpA$_D$, D-adenosine-5′-phosphorimidazolide; NH$_2$pA$_D$, D-adenosine-5′-phosphoramidate; A$_L$pA$_D$, L-adenylyl-D-adenosine; optical density unit measured at 259 nm. See also reference (*1*).
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5. W. M. Huang and P. O. P. Ts'O, *J. Mol. Biol.* **16**, 523 (1966).
6. We thank Dr. F. H. C. Crick for a useful discussion, and Dr. L. Goodman for L-adenosine. Supported in part by grant 13435 from National Institutes of Health.

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Cigarette Smoke: Protection of Alveolar Macrophages by Glutathione and Cysteine

Abstract. *Phagocytosis of bacteria by rabbit alveolar macrophages is inhibited quantitatively by cigarette smoke in vitro. This phagocytoxic effect was abolished by addition of 0.2 to 0.4 micromole of glutathione or cysteine per milliliter of cigarette smoke. Serum protein was required to obtain both the toxic effect of the smoke and the protective action of the sulfhydryl compounds. The protective role of the sulfhydryl agents suggests an oxidant action of the cigarette smoke on these pulmonary cells.*

Among its many actions on lung function, cigarette smoke interferes with the phagocytic activity of alveolar macrophages. When freshly drawn cigarette smoke is introduced into a tissue culture flask containing alveolar macrophages and bacteria, the normally effective bactericidal action of these cells is markedly impaired (*1*). Microscopic examination of stained smears shows a marked reduction in the number of intracellular particles. The effect is quantitative and dose related. Six milliliters of cigarette smoke produced a maximal inhibitory effect. As a hypothetical comparison with human exposure, a comparable dose might be delivered to lungs of average capacity by a minimum of three cigarettes.

Various reducing agents were studied for their possible protective action for the cells against cigarette smoke. Alveolar macrophages were obtained by washout of rabbit lungs, centrifuged, and suspended in Hanks balanced salt solution containing 5 percent autologous rabbit serum. Approximately 10^6 cells were placed in 30-ml plastic tissue-culture flasks in a total volume of 2 ml, which contained also approximately 10^5 *Staphylococcus albus* bacteria and appropriately added chemicals. After all additions were made and the mixture sampled for quantitative bacterial culture, 6 ml of freshly drawn, whole or filtered cigarette smoke was introduced by syringe. The flasks were tightly stoppered, allowed to incubate for 2 hours at 37°C, and again sampled for quantitative bacterial cultures. The change in numbers of viable bacteria in each flask over the 2-hour period was taken as the index of macrophage function. Thus, the method does not distinguish between particle uptake and intracellular destruction.

The percentage of reduction of culturable bacteria seen in control flasks was reproducible within the experiment, although there was variability in the absolute numbers from experiment to experiment. For this reason, conclusions were based on comparison between control and treated flasks within each experiment.

Glutathione, added at the start of the experiment, inhibited the reduction in phagocytosis caused by cigarette smoke (Fig. 1). In control flasks 60 to 80 percent of the bacteria were killed in the 2-hour assay period. Similar phagocytic activity was noted in the flasks containing 2.5 micromoles of glutathione per milliliter, and no smoke.

Complete protection against the phagocytoxic action of the cigarette smoke was gained at a concentration of 2.5 micromoles of glutathione per milliliter of solution in the flask. There was no difference between control and smoked cells in the percent of bacteria killed. This protective effect was absent

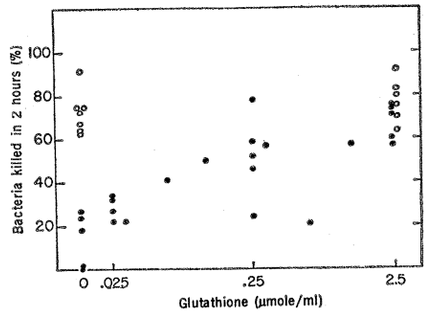

Fig. 1. Effect of glutathione on phagocytoxic activity of cigarette smoke. ○, No smoke added; ●, 6 ml of smoke added.

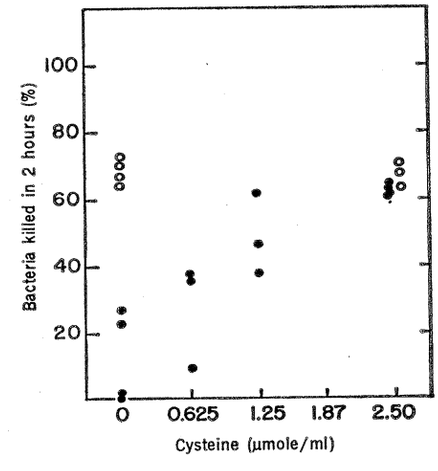

Fig. 2. Dose effect of cysteine on phagocytoxic action of 6 ml of cigarette smoke. ○, No smoke added; ●, 6 ml of smoke added.