clease given by CHEN, YANG and MARTINEZ[6] and the data for the X-ray structural composition of these proteins given by SAXENA and WETLAUFER[4]. 5-point Lagrangian interpolation was used to digitize the basis spectra at intervals of 1 nm. Matrix rank analysis[9] and linear least squares curve fitting[10] were carried out by standard methods. All computations were performed on a Hewlett-Packard 9100B Calculator fitted with a 9101A Extented Memory.

The human erythrocuprein CD-spectra are given in Table I at intervals of 2.5 nm. Rank analysis was performed on a 15×6 matrix whose columns consisted of these spectra and those of myoglobin, lysozyme and ribonuclease[6]. A 5% error matrix was set up as a reasonable estimate. The results of the 5 reduction steps possible with the original data matrix and the corresponding propagated errors are given in Table II. The elements of the principal diagonals of the reduced data and error matrices indicate 3 non-zero rows after reduction. The erythrocuprein and reference protein spectra may therefore be concluded to have the same or a closely similar 3-component basis.

The results of fitting the erythrocuprein spectra with the basis spectra given by the reference proteins are shown in Table III. The goodness of fit for each spectrum is indicated by the low root mean square of the residuals, and by the low standard errors of the estimates for the structural modes. The results suggest that human erythrocuprein has little or no α-helical content and would seem to consist mainly of unordered structure with a smaller proportion of β-structure.

Matrix rank analysis as proposed here can usefully extend the scope and validate the results of conformational analysis from protein CD-spectra. It is important to point out, however, that because of the potentially high signal-to-noise ratio of the spectra in the far ultraviolet and the propagation of errors in the rank analysis, it is difficult to arrive at the right conclusion without an adequate zero-test. A 3-component fit of protein CD-spectra is indicated at the present time, although this may be a simplifying assumption[4-6]. By this argument matrices of reference and analyzed protein spectra with an apparent rank greater than three indicate that the spectra in question do not have the same basis and the set of reference proteins is not appropriately chosen. This type of analysis excludes non-peptide chromophores from consideration[4-6] including protein prosthetic groups. The exclusion is not unreasonable for the prosthetic groups of myoglobin[11] and erythrocuprein[7,12], but it is at best empirical where it can be made[13].

Zusammenfassung. Nachweis mittels vergleichender CD-Spektraluntersuchungen bei einer Auflösung von 2.5 nm von Erythrocuprein, Myoglobin, Lysozym und Ribonuklease, dass menschliches Erythrocuprein keinen oder nur einen geringen Teil mit α-helicoidaler Struktur hat.

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Table III. Structural content of human erythrocuprein preparations as estimated by fitting the CD-spectrum between 205 and 240 nm

	α-Helix (%)	β-Structure (%)	Unordered structure	RMS[a]
Holoprotein	3.0[b] ± 0.5	37.0 ± 0.8	60 ± 1.9	150
Apoprotein	5.9 ± 1	32.9 ± 1.7	61.2 ± 2.2	220
Partial apoprotein	4.8 ± 0.5	33 ± 0.9	62.2 ± 1.2	120

Data points at 1 nm intervals were used. Basis spectra were caluclated from the spectra of myoglobin, lysozyme and ribonuclease[6]. Values include standard error.

[a] Root mean square of residuals for fitted spectrum. [b] Negative with respect to least squares.

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Alkylation of Double-Stranded Ribonucleic Acid with 2-Chloroethylamines

The reaction of 2-haloethylamines with DNA has been extensively investigated[1-4]. With 2-chloroethylamine alkylation initially occurs at both the phosphate ester groups and base moieties, predominantly at the N-7 of guanine. Alkyl groups are then transferred from the phosphate triesters to unsubstituted bases, the alkylated purines are eliminated and hydrolysis of phosphodiester bonds subsequently results in degradation.

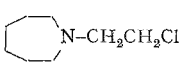
With bifunctional alkylating agents evidence exists for the formation of cross links between double helices[5], between the 2 strands of a single double helix[6-8] and between adjacent bases on the same strand[9].

Using conditions that result in extensive degradation of DNA (Table, Experiments 1, 2 and 3), we have found that the double-stranded RNA isolated from a mycophage[10] can be alkylated by both monofunctional and bifunctional 2-chloroethylamines without degradation.

As for DNA, the primary site of base alkylation in RNA is the guanine N-7 position, although some alkylation can also occur at N-1, N-3 and N-7 of adenine and at N-3 of cytosine[11]. Alkylation at N-7 of guanine is accompanied by the appearance of a chromophore at 280 nm[4] and the relative extent of alkylation can be estimated from the E_{280}/E_{260} ratio.

Alkylation of *ds*-RNA with 2-chloroethyldimethylamine (Exp. 9), 2-chloroethylhexamethyleneimine (Exp. 10), and *bis*-(2-chloroethyl)amine (Experiments 4 and 5) proceeded at similar rates. The products obtained with

Reaction of nucleic acids with 2-chloroethylamines

Expt. No.	Reaction conditions: Nucleic acid	2-Chloroethylamine	Reaction time (h)	Initial pH/ Final pH	E_{280}/E_{260}	Product characteristics: T_m (°C)	Hyperchromicity (%)	pH_m	Sepharose 2B chromatography	Polyacrylamide-gel electrophoresis
1	Calf thymus DNA (Sigma type V)	$(ClCH_2CH_2)_2NH$	19	7.1/5.75	0.573	—	6.8	—	Eluted in bed volume	No high-molecular-weight material remains
2		$(ClCH_2CH_2)_2NH$	116	7.1/4.95	0.644	—	—	—		
3		$(ClCH_2CH_2)_2NCH_3$	116	6.5/3.75	0.711	—	—	—		
4	*ds*-RNA	$(ClCH_2CH_2)_2NH$	17	7.2/5.4	0.500	89	36.0	11.31	K_{av} 0.05; elution pattern identical to that of *ds*-RNA.	Mobility similar to that of *ds*-RNA, but a single band
5		$(ClCH_2CH_2)_2NH$	115	7.2/4.8	0.532	95[a]	28.5	10.83		
6		$(ClCH_2CH_2)_2NCH_3$	19	6.4/4.3	0.538	93[a]	29.0	11.28[b]	Eluted in void volume	Only just moves on to gel
7		$(ClCH_2CH_2)_2NCH_3$	115	6.4/3.45	0.535	95[a]	25.7	11.05[b]		
8		$(ClCH_2CH_2)_2NCH_3$	18	6.6/4.3	0.573	87	36.3	11.43	K_{av} 0.05; elution pattern identical to that of *ds*-RNA	Mobility similar to that of *ds*-RNA, but a single band
9		$ClCH_2CH_2N(CH_3)_2$	22	7.85/6.0	0.514	79	36.5	11.33		
10			22	7.8/6.0	0.500	82	44.4	11.32		

[a] Temperature at which hyperchromicity = 20%; [b] pH at which hyperchromicity = 14.5%.

the monofunctional alkylating agents underwent thermal denaturation at lower temperature and alkali denaturation at lower pH than did *ds*-RNA. The products obtained with the bifunctional alkylating agent also underwent alkali denaturation at lower pH but had elevated melting temperatures (T_m), indicating the presence of interstrand cross linking.

Analysis of the products from Expts. 4, 5, 9 and 10 by gel filtration on Sepharose 2B and polyacrylamide-gel electrophoresis demonstrated that they had the same molecular weight as *ds*-RNA. The enhanced stability of the purine-riboside compared with the purine-deoxyriboside bond at acidic pH is well established[12], and elimination of the alkylated purine followed by degradation of the nucleic acid would not be expected to occur to the same extent in *ds*-RNA as in DNA. However, as for single-stranded polyribonucleotides[13,14], the presence of a free 2′-hydroxyl group would be expected to result in more ready hydrolysis of alkylated internucleotide phosphate linkages. The absence of degradation therefore suggests that in *ds*-RNA the 2′-hydroxyl groups may not be in a favourable conformation for participation in the formation of cyclic phosphate intermediates. In support of this we have found that hydrolysis of *ds*-RNA under a variety of solvent conditions occurs only at pH's above those required for strand separation (M. R. HARNDEN, A. G. BROWN and R. A. VERE HODGE, unpublished work).

As for DNA, alkylation of *ds*-RNA with *bis*-(2-chloroethyl)methylamine (Experiments 6 and 7) proceeded more rapidly than with *bis*-(2-chloroethyl)amine. The alkali denaturation characteristics of the products obtained were, however, quite atypical for *ds*-RNA. Although, as for all of the other alkylation products, strand separation commenced in the region pH 10.2–10.5, the development of hyperchromicity with increasing pH was slower and complete denaturation did not appear to be achieved even at pH 12.4. Under the same conditions separation of *ds*-RNA strands commences at pH 11.2 and is complete at pH 12.2. Gel filtration and polyacrylamide-gel electrophoresis indicated that the *bis*-(2-chloroethyl)methylamine products were highly aggregated, suggesting that with this reagent cross-linking between double helices also occurs.

It has been reported[15] that in aqueous solution *bis*-(2-chloroethyl) methylamine dimerizes, and formation of the dimer is markedly retarded by NaCl. The effect of 75 m*M*-NaCl on its reaction with *ds*-RNA was therefore investigated (Exp. 8). More alkylation occurred than in the absence of NaCl (Exp. 6), but the thermal and alkali denaturation characteristics of the product resembled much more closely those of *ds*-RNA. Gel filtration and polyacrylamide-gel electrophoresis showed that the molecular weight of the product was same as that of *ds*-RNA. The formation of aggregated products could therefore be associated with the ability of the alkylating agent to dimerize.

We have found that alkylation of *ds*-RNA with either monofunctional or bifunctional 2-chloroethylamines yields products with decreased antiviral activity.

Methods. For alkylations the solid 2-chloroethylamine hydrochloride (1 mol/mol of nucleotide for bifunctional alkylating agents and 2 mol/mol of nucleotide for monofunctional compounds) was added to a 2.5 mg/ml solution of the nucleic acid in 2.5 m*M*-NaOH. The pH was immediately determined and reaction carried out at 20 °C for the specified time. The final pH was determined, the NaCl concentration adjusted to 0.15 *M* and the product precipitated with 2 volume of ethanol. For Exp. 8 only the *ds*-RNA was dissolved in a solution 2.5 m*M* in NaOH and 75 m*M* in NaCl.

Various characteristics of the products were determined. The E_{280}/E_{260} ratios were measured; the corresponding value for DNA is 0.543 and that for *ds*-RNA is 0.475. Thermal denaturation characteristics were determined in 15 m*M*-NaCl, 1.5 m*M* trisodium citrate, pH 7. Measurements were carried out in 1 cm cells in a Pye-Unicam SP.800B spectrometer equipped with an SP877 electrically heated cell holder and an SP876 Accuran temperature-programme controller. The solution temperature was elevated by 0.5 °C/min. The hyperchromicity, measured at 260 nm, developed between 30° and 100 °C, is recorded. *Ds*-RNA has T_m 85 °C and hyperchromicity 40.3 %. Alkali denaturation was determined for solutions of the nucleic acid in 0.15 *M*-NaCl at 20 °C by measurement of the immediate hyperchromicity developed with increasing concentrations of NaOH. The maximum hyperchromicity, measured at 260 nm, observed for the nucleic acids under these conditions was 29 % and the recorded pH_m is the value at which the measured hyperchromicity is 50 % of the maximum hyperchromicity. *Ds*-RNA has pH_m 11.75.

For Sepharose 2B chromatography a column 26 cm long 2.5 cm internal diameter, volume approx. 130 ml, was used. The column was eluted in an upward direction with a solution containing 0.15 *M*-NaCl-50 m*M-tris* buffer-5 m*M*-$MgCl_2$-0.002 % NaN_3, pH 7.5, at a flow rate of 0.08 ml/min. For electrophoresis 4 % acrylamide gels containing 0.04 % *bis*-acrylamide were prepared in glass tubes, 4 mm internal diameter. The running buffer was 40 m*M-Tris*-20 m*M*-sodium acetate-2 m*M*-EDTA, pH 7.8. Electrophoresis was carried out at 5 mA/tube for 2 h. The gels were stained with Methylene Blue. *Ds*-RNA moves as 3 sharp closely spaced bands about 1.5 cm into the gel[10].

Zusammenfassung. Nachweis der Alkylierung doppelfädiger RNS mittels 2-Chloräthylaminen in wässeriger Lösung ohne gleichzeitige Hydrolyse der Phosphatesterbindungen zwischen den Nukleotiden, was in starkem Gegensatz zu früheren, bei einfädigen RNS gewonnenen Ergebnissen steht.

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