above, it appears that Mn^{2+} acts on the pigeon liver pyruvate kinase only as a positive effector.

The pigeon liver pyruvate kinase activity can be varied by a small change of Mn^{2+} concentration even when the Mg^{2+} content in the cell is maintained at a constant level. Changes of the Mn^{2+} content in the liver cytoplasm can be postulated, because rat liver mitochondria are able to accumulate Mn^{2+} from a surrounding solution[13]. However, the lack of knowledge of the actual ion environment of the enzyme in the cell precludes the exact valuation of the phenomena in vivo.

Riassunto. Mn^{2+} and Mg^{2+} attivano la piruvato cinasi di fegato di piccione in maniera distinta. In presenza di basse concentrationi di fosfoenolpiruvato Mn^{+} é piú efficace di Mg^{2+} ed é attivatore dell'enzima saturato da Mg^{2+}. Piruvato cinasi (EC 2.7.1.40).

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Conformational Analysis of Proteins from Circular Dichroism Spectra with Reference to Human Erythrocuprein

The interpretation of protein conformation from circular dichroism (CD) spectra has attracted much attention[1-5]. It is assumed that the far ultraviolet CD-spectrum has a basis consisting of the spectra of the α-helical, β-structural and unordered conformations, and is given by a linear combination of these spectra. Although poly-α-amino acid CD-spectra give good approximations in certain cases[1,3], it is doubtful whether these spectra can form a true basis for protein spectra[4-6]. The use of basis spectra calculated from the CD-spectra of reference proteins whose structural composition has been determined by X-ray analysis has therefore been proposed[4-6]. The reservations in this approach have been pointed out[4-6]. We here draw attention to the need to assume that the set of reference and analyzed protein spectra have the same basis. This assumption is amenable to confirmation by rank analysis of the matrix of reference and analyzed protein spectra. For a valid 3-component fit of the analyzed spectra this matrix should have a rank of three. We propose this matrix rank analysis prior to curve fitting of protein CD-spectra with calculated basis spectra as in the computations reported here on CD-spectra of human erythrocuprein.

The profiles of the analyzed human erythocuprein CD-spectra have been reported previously[7]. The spectra were digitized at intervals of 1 or 2.5 nm and the data were smoothed by a quadratic 5-point least squares approximation[8]. Basis spectra were calculated from the CD-spectra of myoglobin, lysozyme and ribonu-

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Table I. CD-spectra of human erythrocuprein preparations

Wavelength (nm)	Mean residue ellipticity (deg. cm²/d mol)		
	Holoprotein	Apoprotein	Partial apoprotein[a]
205	−3410	−2700	−3030
207.5	−3740	−3280	−3310
210	−3600	−3280	−3200
212.5	−3150	−2980	−2810
215	−2560	−2630	−2400
217.5	−1920	−2160	−1920
220	−1230	−1600	−1480
222.5	−530	−1070	−1050
225	130	− 630	− 640
227.5	500	− 420	− 360
230	620	− 250	− 140
232.5	550	− 230	− 70
235	450	− 240	− 50
237.5	370	− 240	10
240	360	− 160	70

[a] Contained 60% of original copper and no zinc.

Table II. Results of rank analysis of a matrix of the CD-sprectra of Table I and the spectra of myoglobin, lysozyme and ribonuclease[6]

Reduced data matrix[a]

−24850	−9580	−7810	− 530	−1070	−1050
0	−7003	−4260	−3111	−2097	−2438
0	0	2960	1471	783	936
0	0	0	− 634	− 621	− 379
0	0	0	0	− 394	− 12
0	0	0	0	0	− 95

Reduced error matrix

1243	479	391	27	54	53
0	776	577	172	145	160
0	0	616	298	208	239
0	0	0	796	504	581
0	0	0	0	855	688
0	0	0	0	0	491

[a] First 6 rows.

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