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Dedicated to Professor Ionel Haiduc
on the occasion of his 70th anniversary

OPTICAL ROTATORY DISPERSION OF SOME HUMAN SERUM ALBUMIN CONFORMERS

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Optical rotatory dispersion of some human serum albumin (HSA) conformers was investigated in order to determine the differences in their α-helix content. Various conformers were obtained using thermal defolding-refolding cycle. The obtained results for HAS are in agreement with the results for bovine serum albumin (BSA) conformers and with the supposition that the conformers obtained from a slow refolding have a larger α - helix content compared with those obtained after more rapid processes.

INTRODUCTION

The optical rotation angle is very sensitive to the molecular structure neighbouring of each asymmetry centre.[1] Due to the fact that the asymmetry centres are identical (up to the radical R) in the albumin macromolecule, the contribution of each centre depends not only upon its own structure but also upon the neighbouring groups.[2] It is obvious that the active centres at the ends of the polypeptidic chain will exhibit rotation conditions of the polarized light plane different to some extent from the ones produced by the interior groups. It is also obvious that in the rotation of the radiation polarization plane interaction effects are observed in other physico-chemical processes.

Continuing our previously research concerning the albumin conformers[3], obtained in defolding – refolding cycles[4], in this paper are studied the optical activity of some HSA conformers.

RESULTS AND DISCUSSION

The concentration c in human serum albumine being expressed in grams/mL of solution, the following relation of the specific rotatory power was used:

$$[\alpha]_{\lambda}^{t} = \frac{\alpha}{\ell c}$$

where:

α - the rotating angle of the polarization plane,

ℓ - the length, in decimeters, of the solution column.

For the specific rotatory power, Drude's theory[5] lead to the following relation:

$$[\alpha] = \frac{a_0 \lambda_0^2}{\lambda^2 - \lambda_0^2}$$

were:

a_0 - a constant,

λ_0 - the wave length associated to substance absorbtion,

λ - the wave length at which the rotating angle was determined.

The terms of Drude's relation can regroup themselves, leading to a product with linear dependence $\lambda^2 [\alpha]$ upon [α]:

$$\lambda^2 [\alpha] = \lambda_0^2 [\alpha] + a_0 \lambda_0^2$$

Table 1 shows the values [α] for a concentration of 2 mg mL^{-1} and a temperature of 21°C.

A continuous decrease can be observed for sample 1 to sample 4, but sample 5 presents an increase and then a constant value of [α] in the visible domain.

After linearizing Drude's relation with reference to the normal rotatory dispersion, the following results (table 2) were obtained (R - the correlation coefficient).

Table 1

The values of specific rotatory power - [α] for wavelengths λ

λ (μ) Sample	λ_1 0.366	λ_2 0.406	λ_3 0.436	λ_4 0.546	λ_5 0.578
1	220.312	159.375	134.371	82.818	75.003
2	264.061	192.190	160.949	98.446	89.066
3	256.250	181.252	151.563	92.193	84.375
4	228.129	165.628	139.067	85.946	76.566
5	234.376	265.625	296.876	296.876	296.876

Table 2

The values of λ_0^2 and $a_0 \lambda_0^2$ from Drude's relation

Sample	λ_0^2	$a_0 \lambda_0^2$	R
1	0.030	22.122	0.934
2	0.034	25.942	0.931
3	0.036	24.168	0.945
4	0.032	22.689	0.954
5	0.849	- 172.380	0.819

From table 2 it may be concluded that Drude's relation describes with enough precision a phenomenon of normal rotatory dispersion only for samples 1- 4. Using this relation for experimental data an average value of $\lambda_0 = 201$ nm is obtained, at the middle interval (λ = 180 – 220 nm) of the very intense albumin absorbtion band in UV. But, the correlation coefficient values in this case, suggest the existence of the interaction with another weak but very wide one with the maximum at λ = 283 nm. It was assumed that the effect of this weak absorption can manifest it self in the rotatory dispersion up to a close UV and even in the violet-blue region of the visible spectrum. This fact justify the utilisation of Drude's abnormal ecuation for only these λ.

In order to verify Drude's relation for abnormal dispersion the following relations were used:

$$[\alpha] = \frac{a_0\lambda_0^2}{\lambda^2 - \lambda_0^2} + \frac{b_0\lambda_0^4}{(\lambda^2 - \lambda_0^2)^2}$$

$$[\alpha](\lambda^2 - \lambda_0^2) = a_0\lambda_0^2 + b_0\lambda_0^4 \frac{1}{\lambda^2 - \lambda_0^2} = A + B\frac{1}{\left(\lambda^2 - \lambda_0^2\right)}$$

The values of constant a_0 and b_0 from Drude's relation for abnormal dispersion are prezentated in table 3. If the value λ_0 previously obtained was used for this treatment of data, a linear representation was obtained leading to b_0 values which evidentiate changes in the albumin helicoidal contribution in the studied conformers.

It is obvious that the value of a_0 remains quasiconstant. The value of b_0, which indicates the content of α – helix, increase from sample 4 to 1, the α - helix content being higher in sample 1, which is a natural unheated sample.

$$\alpha\text{ - helix} \xleftarrow{B_1 > B_2 > B_3 > B_4}$$

The obtained results for HSA are in agreement with those for BSA[3], some differences explaining by the composition in aromatic aminoacids in HAS and BSA[6] (table 4). The anomality effect supposed to be due to the weak albumin absorbtion band at λ = 283 nm, being evident that the π electronics structures (different for HAS and BSA) are responsible for this anomality.

Table 3

The value of constants A and B from Drude's relation

Sample	a_0	b_0	R
1	635.96	433.33	0.9940
2	681.61	275.52	0.9836
3	642.87	59.41	0.9897
4	693.64	18.75	0.9580

Table 4

The number of aromatic aminoacids in HAS and BSA

Aromatic aminoacids	HSA	BSA
Phenylalanine	31	27
Tyrosine	18	20
Tryptophan	1	2
Histidine	16	17
Total number	66	66

EXPERIMENTAL

Substances: HSA (Kamada, Israel) was used without further purification.

Method and apparatus: In the study of thermic denaturation there were used solutions of 1mg/mL of HSA contained in Nalgere Cryovial 2 mL tubes. They were then slowly heated in a water bath up to 70°C, maintaining 15 min this temperature.

The initial solution sample was noted with 1 and used as the reference case. The renaturation process was accomplished through different thermic regime noted as follows: 2 – slowly cooled; 3 – cooled at environment temperature; 4 – cooled at 5 ^{0}C; 5 – thermic shock at –15 ^{0}C.

The optical activity was studied with a POLAMAT A ZEISS JENA polarimeter providing a 0,005° precision in reading the rotating angle. The device with a set of interferential filters allows spectropolarimetric determinations measurements at five wave lengths: 366, 406, 436, 546 and 578 nm.

The capillary microtube used has 0,64 dm in length, approximately 2mL volume, with quartz windows, its temperature being maintained at a certain value by connecting the device to a thermostat (±0,05°) which ensures the thermic agent flow.

CONCLUSION

The obtained optical rotatory dispersion results for HSA are in agreement with the results for BSA. The HAS conformers obtained from a slow refolding process have a larger α - helix content compared with those obtained after more rapid processes Thermic shock emphasize the structural disorder of the macromolecule.

REFERENCES

1. C. Tanford, ,,Physical Chemistry of Macromolecules", Wiley, New York, 1961.
2. A. Fersht, ,,Structure and mechanism in protein science", Freeman, New York, 2002.
3. V. Chiosa, C. Pantazica, V. Em. Sahini, *Rev. Roum. Chem*., **2005**, *50*, 503-506.
4. V. Em. Sahini, G. Ioniță, C. Postolache, C.Tilimpea and D. Dinu, *J. Plan. Chromatogr*., **2003**, *4*, 308-310.
5. I. G. Murgulescu and V. Em. Sahini, ,,Introducere în chimia fizică", Ed. Academiei, Bucharest, 1978
6. T. Peters Jr,, All about albumin", Academic Press, New York, 1996.