# Prediction structure type for human leukocyte interferon subtype A from circular dichroism

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Vacuum UV circular dichroism studies were carried out on human leukocyte interferon subtype A. The secondary structure analysis for the CD spectrum shows 59% α-helix, 16% antiparallel β-sheet, no parallel β-sheet, 18% β-turns and 13% other structures. The analysis of the CD features for the prediction of tertiary structural class reveals that it is an all-α type protein.

*Circular dichroism* *Protein secondary structure* *Interferon* *Tertiary fold*

## 1. INTRODUCTION

Interferon has attracted a great deal of attention, not only because of its antiviral effect, but also because of its other biological activities [1,2]. It is not a single protein but refers to a large family of proteins varying from species to species and present in multiple forms even within a single species [2]. These classes have been defined for human interferon and have been designated as leukocyte interferon ($\alpha$), fibroblast interferon ($\beta$) and immune interferon ($\gamma$). Also, different members of the alpha family have been isolated and their amino acid sequences have been predicted from DNA which show related primary structures [3–6]. To understand the mechanism of interferon action it is necessary to know its three-dimensional structure. Though its crystal structure has not yet been solved, the three-dimensional structure has been predicted from the amino acid sequence to be four up-and-down antiparallel $\alpha$-helical bundles [7]. However, Raman [8] and circular dichroism (CD) studies [9] in solution have produced conflicting predictions for secondary structure. In this paper we report vacuum UV CD studies of human leukocyte interferon A (IFN-$\alpha$A). Our results show that it is predominantly an $\alpha$-helical protein with $59 \pm 5\%$ $\alpha$-helix, and $16 \pm 5\%$ antiparallel $\beta$-sheet, in close agreement with some of the predictions made from amino acid sequence. Further, we predict the tertiary structure of interferon from the positions and the relative magnitudes of the bands and crossover regions in the CD spectra.

## 2. MATERIALS AND METHODS

IFN-$\alpha$A was prepared from extracts of *Escherichia coli* [10] by a modification of the procedure in [11] using an immobilized antibody column (either LI-8, provided by Hoffmann–La Roche, or NK-2, purchased from Celltech). The protein was greater than 98% pure as determined by Coomassie blue and silver staining of SDS polyacrylamide gels loaded both in the presence and absence of reducing agents.

The protein had a specific activity of $2 \times 10^8$ U/mg by both the antiviral activity and an immunosorbant assay. The antiviral activity was assessed by a microtiter plate cytopathic effect assay using vesicular stomatitis virus on cultured bovine kidney cells [10]. The enzyme-linked immunosorbant assay utilized two monoclonal anti-interferon antibodies: free LI-9 and peroxidase-linked LI-1. The use of these monoclonal an-

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tibodies in an interferon radioimmunoassay has been described in [12]; both antibodies were kindly provided by Hoffman–La Roche, Inc.

The CD sample was prepared by dialyzing 1.25 mg of IFN-$\alpha$A (1.6 mg/ml) against 2 × 1 l of glass distilled $H_2O$ for 15 h. It was lyophilized and dissolved in 1.0 ml of 0.1 M sodium phosphate, pH 6.5, 20% $CH_3CN$ to yield a final concentration of 0.98 mg/ml. The UV spectrum from 400 to 240 nm showed insignificant scattering from the sample. The samples were assayed before and after preparation with no differences in specific activity.

The CD spectrum was measured on a vacuum UV CD spectrophotometer [13] using techniques described elsewhere [14]. The extinction coefficient at 190 nm was determined to be 9200 $l \cdot mol^{-1} \cdot cm^{-1}$. Digitized CD data were analyzed for secondary structure using the method developed by authors in [14]. The important CD features were also analyzed and compared with the results representing four different tertiary structural types [15], in order to predict the tertiary structure.

## 3. RESULTS AND DISCUSSION

The CD spectrum of interferon is shown in fig.1. The spectrum displays two negative bands at 217–219 nm and 209 nm and an intense positive band at 191 nm which are characteristics of the $\alpha$-helical structure. Also observed in the CD spectrum of interferon is a positive band around 232–234 nm pushing into the intense and broad

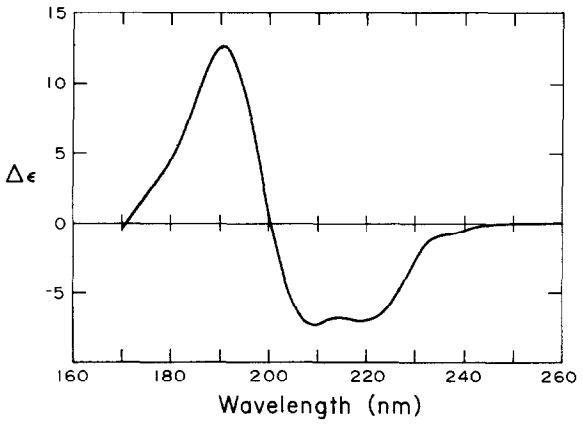


Fig.1. The CD spectrum of human leukocyte interferon-$\alpha$A.

negative CD, consistent with the observations made by authors in [9]. The origin of this positive band is not known, though it may be due to the contributions from aromatic residues [9].

The spectrum was analyzed for secondary structure using 5 basis CD vectors and their corresponding structure vectors derived from 16 sample proteins [14]. The sum of fractions of secondary structure was not constrained to equal 1.0. The analysis shows that the protein contains 59% $\alpha$-helix, 16% antiparallel $\beta$-sheet, 18% $\beta$-turns and 13% other structures (table 1) and shows no parallel $\beta$-sheet in the protein.

Proteins have been broadly classified into four major tertiary structural classes [16]: all-$\alpha$, $\alpha+\beta$, $\alpha/\beta$ and all-$\beta$ types. All-$\alpha$ is defined as predominantly $\alpha$-helical structures; $\alpha+\beta$ as rich in both $\alpha$-helix and $\beta$-sheet but with these structures separated; $\alpha/\beta$ as both $\alpha$-helix and $\beta$-sheet, but mixed or occurring in alternating segments; and all-$\beta$ as predominantly $\beta$-sheet. It is now possible to predict any one of these tertiary structures from the analysis of the CD characteristics [15]. The CD of interferon is characteristic of $\alpha$-helical structure, and the short wavelength crossover at 171 nm puts this protein in the all-$\alpha$ category [15].

The secondary structure of IFN-$\alpha$s has been predicted from limited amino acid sequence data [7,17]. Using an algorithm developed in [18], a more comprehensive set of predicted structures were determined for a number of IFN-$\alpha$s based on amino acid sequences derived from cDNA [19]. An average prediction based on eight IFN-$\alpha$ sequences agrees well with our CD results (63% helix compared with 59% from the CD). In addition, authors in [19] tested the predicted structure against hydrophobicity calculations and limited proteolytic cleavage of assumed exposed regions. These comparisons were consistent with the predicted structures. We used the same algorithm to repeat the secondary structure prediction of an average structure based on the primary sequence of eleven IFN-$\alpha$ cDNA and genomic clones [3–6]. These calculations are given in table 1 and agree with those in [19], which did not report the values for structure elements other than $\alpha$-helix. The calculated average structure along with that of IFN-$\alpha$A, also shown in table 1, agree well with those determined by our CD results.

Although the predictions in [7] and [17] were

Table 1

Secondary structure analysis of interferon from amino acid sequence and CD

| Prediction method | $\alpha$-Helix | $\beta$-Sheet | Turns | Others (or coil) |
|---|---|---|---|---|
| Lim[a] | 0.48 | 0.09 | – | 0.43[b] |
| Chou and Fasman[a] | 0.47 | 0.14 | 0.19 | 0.20 |
| Robson and co-workers[a] | 0.51 | 0.11 | 0.14 | 0.24 |
| Chou and Fasman[c] | 0.55 | 0.16 | 0.10 | 0.19 |
| Our CD work | 0.59 | 0.16 | 0.18 | 0.13 |
| Our calculation IFN-$\alpha$A[d] | 0.64 | 0.17 | 0.08 | 0.11 |
| Our calculation average[e] | 0.63 | 0.16 | 0.12 | 0.09 |

[a] Calculated from the results in [7]
[b] Includes both turns and coil
[c] Taken from Hayes [17]
[d] Secondary structure prediction based on Robson method for IFN-$\alpha$A
[e] Secondary structure prediction of an average of 11 IFN-$\alpha$ sequences using the Robson method

from limited sequence data, these are also included in table 1. Using the Chou and Fasman method, the authors in [17] predicted 55% $\alpha$-helix and 16% $\beta$-sheet (table 1). Three different methods (Chou and Fasman, Lim, and Robson group) were used in [7] to predict the secondary structure of interferon. The predictions of $\alpha$-helix and $\beta$-sheet vary from 47 to 51% and 9 to 14%, respectively, for the same protein depending upon the method used for the prediction. It is interesting that their predictions of $\alpha$-helix and $\beta$-turns by the Chou and Fasman method are different from Hayes. However, the $\beta$-sheet content is similar for both and agrees with our predictions. Taken collectively, both our predicted values and the others are consistent with the experimentally derived values presented in this paper.

In other work, the author in [8] has predicted 76 ± 5% $\alpha$-helix and 7 ± 3% $\beta$-sheet from his Raman studies. A 40–45% $\alpha$-helix from CD results was predicted in [9] but later the authors obtained 70 ± 5% $\alpha$-helix when they used freshly prepared IFN-$\alpha$A. All of the results to date therefore support the idea that IFN-$\alpha$ represents a family of proteins with high $\alpha$-helical potential.

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