Structure of Human Serum Albumin

We have reported the crystal structure of human serum albumin (HSA) at a resolution of 6.0 Å by the method of multiple isomorphous replacement (*1*). A different quaternary arrangement of the six subdomains than that reported at low resolution has become apparent at 4 Å resolution (*2*). The change in the electron density involves connecting the previously labeled amino- and carboxyl-terminal helices through the symmetry operation (*Y, X,* 1-*Z*) together with a concomitant formation of new termini (Fig. 1A). This connection was not revealed in the electron density at 6.0 Å mainly because of series termination effects that are usually more pronounced at lower resolution. The attachment of the tail (subdomains IIB, IIIA, and IIIB) to the head (subdomains IA, IB, and IIA) of the molecule differs from that originally proposed and requires relabeling of certain subdomains (Fig. 1B). Further evidence supports this quaternary arrangement. The subdomains assume a heart shape, which agrees with the dark-field electron micrograph images of the genetically related human and bovine α-fetoproteins (AFP) (*3*). Domains I, II, and III may be superimposed (Fig. 1C), which is consistent with the homology within the amino acid sequence. The major ligand binding regions are identified within subdomains IIA (previously labeled IA) and IIIA, which is consistent with the competitive drug displacement experiments (*4*) and

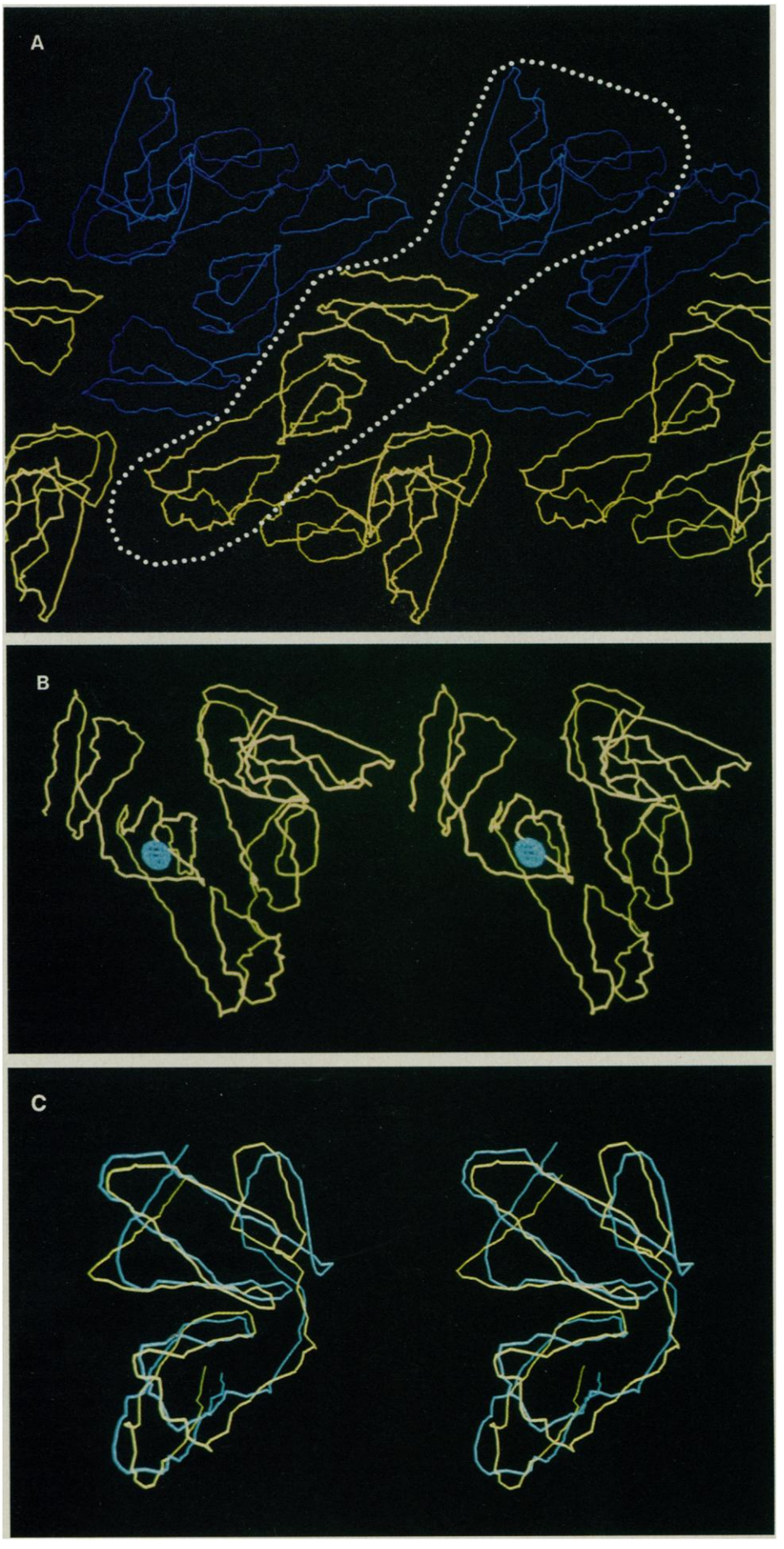

Fig. 1. (**A**) Illustration of the close packing of the HSA molecules along columns in the (*a*/2, *b*/2) direction viewed perpendicular to the *c*-axis. The individual HSA molecules are shown in yellow and blue. An outline of the quaternary arrangement of the subdomains reported at 6.0 Å is illustrated. (**B**) Stereoview of a simplified tracing (not α-carbons) representing the convolution of electron density within a molecule of HSA based on the interpretation of the 4.0 Å electron density. The subdomains from left to right are IIIB, IIIA, IIB, IIA, IB, and IA. The difference density illustrating the major binding location for ibuprofen within IIIA is shown. The width of the molecule from the amino- to the carboxyl-terminal domain is ~82 Å, and the maximum dimensions of the molecule from the apex of the heart to the amino- and carboxyl-terminal domains are approximately 83 and 70 Å, respectively. The depth of the molecule is roughly 30 Å. (**C**) A stereoview of independent tracings of the electron density within domains II (yellow) and III (blue) superimposed to illustrate the structural homology.

the reported acetylation of Lys^{199} by aspirin (*5*). Distances measured between various amino acids and bound ligands to HSA and bovine serum albumin obtained from spectroscopic data from a variety of fluorescence and resonance energy-transfer studies (*6*) support the AFP-type configuration. Finally, proteolytic cleavage of HSA would produce two halves of the molecule that could reassociate in solution, thereby restoring the binding properties of the intact albumin (*7*). Thus this quaternary arrangement is in more general agreement with the chemistry and molecular biology of HSA.

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8 May 1990; accepted 24 May 1990

Deprenyl and the Progression of Parkinson's Disease

In the report by James W. Tetrud and J. William Langston on "The effect of deprenyl (Selegiline) on the natural history of Parkinson's disease" (*1*), the authors conclude that deprenyl treatment retards the progression of Parkinson's disease. They say they arrive at this conclusion because they found that a group of placebo-treated patients required L-dopa treatment sooner than a group of deprenyl-treated subjects; however these investigators assessed the apparent severity of the illness while the patients in the deprenyl group were receiving deprenyl. Thus one group was receiving treatment with a monoamine oxidase inhibitor, which increases dopamine levels in the brain, while the other group was receiving no active treatment at the time of the assessment.

It seems quite probable that the deprenyl group would appear to have progressed less compared to the placebo group simply because they were receiving deprenyl and thus displaying fewer symptoms. The authors attempt to deal with this possible confounding factor by showing that at the time the deprenyl group required L-dopa for symptom control, they did not get worse when the deprenyl was discontinued. This, however, is not an adequate demonstration that deprenyl was not producing therapeutic benefits at earlier assessment points when the Parkinson's was less severe (that is, when the deprenyl group appeared to have an advantage over the placebo group).

In order for Tetrud and Langston to have correctly arrived at their conclusions, the subjects should have been withdrawn from deprenyl at each assessment point and the assessment carried out after deprenyl wash-out, so that Parkinson's status could have been assessed in an untreated state. Alternatively the placebo group could have been treated with deprenyl before each assessment so that the same level of monoamine oxidase inhibition could be achieved in each group.

In the absence of a comparable assessment of the two groups it is not justifiable to conclude that deprenyl retards the progression of Parkinson's disease, however attractive this possibility is from a theoretical standpoint.

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17 August 1989; accepted 4 May 1990

I read with interest the report of Tetrud and Langston (*1*) on the effect of deprenyl on the natural history of Parkinson's disease. The data clearly demonstrate an effect on the clinical status of treated patients. I take issue, however, with the authors' conclusion that deprenyl slows the progression of Parkinson's disease. The authors suggest several mechanisms for the observed effect, all of which imply a greater preservation of nigral neurons. This preservation needs to be more clearly demonstrated.

The end point of the study was the patients' requirement for L-dopa therapy. Deprenyl may forestall the need for L-dopa by means of its synaptic effect, without protecting nigral neurons. Deprenyl is a monoamine oxidase–B inhibitor, used in Europe for more than a decade as an adjunct in the treatment of Parkinson's disease. Patients taking deprenyl commonly require a downward adjustment of their L-dopa dose by 20 to 30%. It may be that deprenyl has a modest effect as *primary therapy* in early Parkinson's disease. Perhaps it acts to increase the synaptic persistence of endogenously released dopamine. If so, it might delay the requirement for supplemental L-dopa by some months. (This is an issue that will also need to be addressed by the DATATOP study cited by the authors.)

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21 August 1989; accepted 4 May 1990

Response: Both Sudarsky and Friedhoff raise the important issue of whether or not a symptomatic effect of deprenyl, rather than a slowing of the disease process, could account for the results of our study. As pointed out by Sudarsky, since deprenyl is an inhibitor of monoamine oxidase, it might increase the synaptic persistence of endogenously released dopamine, thereby leading to some degree of symptomatic improvement. To assess this possibility, two steps were taken in our study. First, patients were carefully reevaluated 1 month after the study drug was started to see if they had improved compared to their baseline evaluation (this would have indicated a symptomatic effect). At this 1-month "wash-in" evaluation, no symptomatic improvement was observed. Even more important, when patients reached end point (that is, the need for L-dopa therapy), the study drug was stopped for an entire month ("wash-out"), after which they were carefully reevaluated. Had deprenyl been providing a symptomatic effect later in the course of treatment, one would have expected deterioration, and none was observed. While it could be argued that the wash-out period might have been too short, we are unaware of any antiparkinsonian drugs that provide an unremitting symptomatic effect for as long as 1 month after they are discontinued.

Friedhoff raises the interesting point that