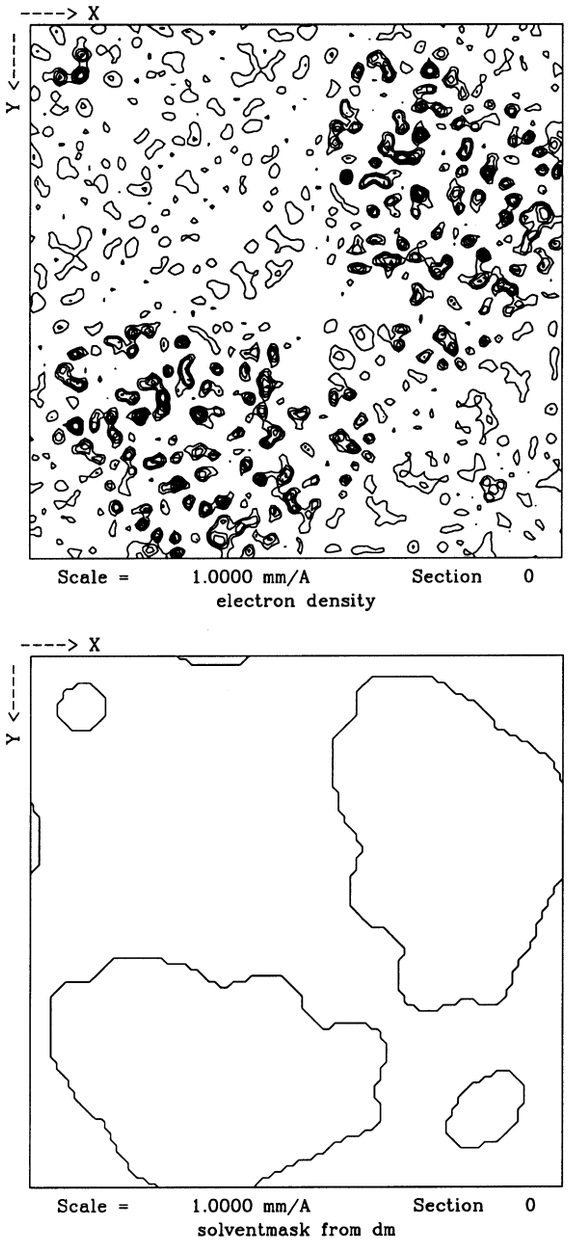

Fig. 15.1.2.2. Solvent mask determined from a map by Wang's method.

density, which is the sum of the absolute values of all density points within a small box.

15.1.2.1.2. *The automated convolution method for molecular-boundary identification*

Wang (1985) suggested an automated convolution method for identifying the solvent region which has achieved widespread use. His method involved first calculating a truncated map:

$$\rho_{\rm trunc}(\mathbf{x}) = \begin{cases} \rho\mathbf{x}, & \rho(\mathbf{x}) > \rho_{\rm solv} \\ 0, & \rho(\mathbf{x}) < \rho_{\rm solv} \end{cases}. \qquad (15.1.2.1)$$

The electron density is simply truncated at the expected solvent value, $\rho_{\rm solv}$; however, since the variations in density in the protein region are much larger than the variations in the solvent region, it is generally only the protein region which will be affected. Thus, the mean density over the protein region is increased. Similar results may be obtained using the mean-squared difference of the density from the expected solvent value.

A smoothed map is then formed by calculating at each point in the map the mean density over a surrounding sphere of radius R. This operation can be written as a convolution of the truncated map, $\rho_{\rm trunc}$, with a spherical weighting function, $w(\mathbf{r})$,

$$\rho_{\rm ave}(\mathbf{x}) = \sum_{\mathbf{r}} \mathrm{w}(\mathbf{r})\rho_{\rm trunc}(\mathbf{x}-\mathbf{r}), \qquad (15.1.2.2)$$

where

$$w(\mathbf{r}) = \begin{cases} 1-|\mathbf{r}|/R, & |\mathbf{r}| < R \\ 0, & |\mathbf{r}| > R \end{cases}. \qquad (15.1.2.3)$$

Leslie (1987) noted that the convolution operation required in equation (15.1.2.2) can be very efficiently performed in reciprocal space using fast Fourier transforms (FFTs),

$$\rho_{\rm ave}(\mathbf{x}) = \mathscr{F}^{-1}\{\mathscr{F}[\rho_{\rm trunc}(\mathbf{x})]\mathscr{F}[w(\mathbf{r})]\}, \qquad (15.1.2.4)$$

where $\mathscr{F}$ denotes a Fourier transform, and $\mathscr{F}^{-1}$ represents an inverse Fourier transform.

The Fourier transform of the truncated density can be readily calculated using FFTs. The Fourier transform of the weighting function can be calculated analytically by

$$g(s) = \mathscr{F}[w(\mathbf{r})] = \frac{3[\sin(2\pi Rs) - 2\pi Rs\cos(2\pi Rs)]}{(2\pi Rs)^3} - \frac{3\{4\pi Rs\sin(2\pi Rs) - [(2\pi Rs)^2 - 2]\cos(2\pi Rs) - 2\}}{(2\pi Rs)^4}, \qquad (15.1.2.5)$$

where

$$s = 2\sin\theta/\lambda.$$

Therefore, the averaging of the truncated electron density by a spherical weighting function can be achieved by two FFTs. This greatly reduced the time required for calculating the averaged density. Other weighting functions may be implemented by the same approach.

A cutoff value, $\rho_{\rm cut}$, is then calculated, which divides the unit cell into two portions occupying the correct volumes for the protein and solvent regions. All points in the map where $\rho_{\rm ave}(\mathbf{x}) < \rho_{\rm cut}$ can then be assumed to be in the solvent region. A typical mask obtained from an MIR map by this means, and the modified map, are shown in Fig. 15.1.2.2.

The radius of the sphere, R, used in equation (15.1.2.3) for the averaging of electron densities is generally around 8 Å. The molecular envelope derived from such an averaged map tends to lose details of the protein molecular surface. Paradoxically, a large averaging sphere is required for the identification of the protein–solvent boundary based on the difference between the mean density of the protein and solvent, which is very small and can only be distinguished when a sufficiently large area of the map is averaged. Abrahams & Leslie (1996) proposed an alternative method of molecular-boundary identification that uses the standard deviation of the electron density within a given radius relative to the overall mean at every grid point of a map. The local-standard-deviation map is the square root of a convolution of a sphere and the squared map, which can be calculated in reciprocal space in a similar way to the procedure described in equations (15.1.2.4) and (15.1.2.5) as proposed by Leslie (1987). By integrating the histogram of the local-standard-deviation map, the cutoff value of the local standard deviation corresponding to the solvent fraction can be calculated. Using this procedure, a molecular envelope that contains more details of the protein molecular surface can be obtained, since the radius of the averaging sphere can be as low as 4 Å (Abrahams & Leslie, 1996).

references